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(54) Title: MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

(57) Abstract: Modified antibodies, or antigen-binding fragments thereof, to the extracellular domain of human prostate specific membrane antigen (PSMA) are provided. The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. Pharmaceutical compositions including the aforesaid antibodies, nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments are also disclosed. Methods of using the antibodies of the invention to detect human PSMA, or to ablate or kill a PSMA-expressing cell, e.g., a PSMA-expressing cancer or prostatic cell, either *in vitro* or *in vivo*, are also provided.

MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. provisional application number 60/295,214 filed on June 1, 2001, 60/323,585 filed on September 20, 2001, and 60/362,810 filed on March 8, 2002, the contents of all of which are incorporated herein by reference. This invention was made with government support under Department of Defense Grant number DAMD17-98-1-8594. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, e.g., modified, e.g., deimmunized, antibodies, to the extracellular domain of human prostate specific membrane antigen (PSMA) and their uses in treating, preventing, and diagnosing prostatic disorders and cancers.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is one of the most common causes of cancer deaths in American males. In 1999, approximately 185,000 new cases were diagnosed and 37,500 died of this disease (NCI SEER data). It accounts for about 40% of all cancers diagnosed in men. A male born in the U.S. in 1990 has approximately a 1 in 8 likelihood of being diagnosed with clinically apparent prostate cancer in his lifetime. Even prior to the recent increase in incidence, prostate cancer was the most prevalent cancer in men (Feldman, A.R. et al. (1986) *NEJM* 315:1394-7).

[0003] There is currently very limited treatment for prostate cancer once it has metastasized (spread beyond the prostate). Currently, systemic therapy is limited to various forms of androgen (male hormone) deprivation. While most patients will demonstrate initial clinical improvement, virtually inevitably, androgen-independent cells develop. Endocrine therapy is thus palliative, not curative. In a study of 1387 patients with metastatic disease detectable by imaging (e.g., bone or CT scan), the median time to objective disease progression (excluding biochemical/PSA progression) after initiation of hormonal therapy (i.e., development of androgen-independence) was 16-48 months (Eisenberger M.A., et al. (1998) *NEJM* 339:1036-42). Median overall survival in these patients was 28-52 months from the onset of hormonal treatment (Eisenberger M.A., et al. (1998) *supra.*). Subsequent to developing androgen-independence, there is no effective standard therapy and the median duration of survival is 9-12

months (Vollmer, R.T., et al. (1999) *Clin Can Res* 5: 831-7; Hudes G., et al., (1997) *Proc Am Soc Clin Oncol* 16:316a (abstract); Pienta K.J., et al. (1994) *J Clin Oncol* 12(10):2005-12; Pienta K.J., et al. (1997) *Urology* 50:401-7; Tannock I.F., et al., (1996) *J Clin Oncol* 14:1756-65; Kantoff P.W., et al., (1996) *J. Clin. Oncol.* 15 (Suppl):25:110-25). Cytotoxic chemotherapy is poorly tolerated in this age group and generally considered ineffective and/or impractical. In addition, prostate cancer is relatively resistant to cytotoxic agents. Thus, chemotherapeutic regimen has not demonstrated a significant survival benefit in this patient group.

[0004] For men with a life expectancy of less than 10 years, watchful waiting is appropriate where low-grade, low-stage prostate cancer is discovered at the time of a partial prostatectomy for benign hyperplasia (W.J. Catalona, (1994) *New Engl. J. Med.*, 331(15):996-1004). Such cancers rarely progress during the first five years after detection. On the other hand, for younger men, curative treatment is often more appropriate.

[0005] Where prostate cancer is localized and the patient's life expectancy is 10 years or more, radical prostatectomy offers the best chance for eradication of the disease. Historically, the drawback of this procedure is that most cancers had spread beyond the bounds of the operation by the time they were detected. However, the use of prostate-specific antigen testing has permitted early detection of prostate cancer. As a result, surgery is less extensive with fewer complications. Patients with bulky, high-grade tumors are less likely to be successfully treated by radical prostatectomy.

[0006] After surgery, if there are detectable serum prostate-specific antigen concentrations, persistent cancer is indicated. In many cases, prostate-specific antigen concentrations can be reduced by radiation treatment. However, this concentration often increases again within two years.

[0007] Radiation therapy has also been widely used as an alternative to radical prostatectomy. Patients generally treated by radiation therapy are those who are older and less healthy and those with higher-grade, more clinically advanced tumors. Particularly preferred procedures are external-beam therapy which involves three dimensional, conformal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy.

[0008] For treatment of patients with locally advanced disease, hormonal therapy before or following radical prostatectomy or radiation therapy has been utilized. Hormonal therapy is the main form of treating men with disseminated prostate cancer. Orchiectomy reduces serum testosterone concentrations, while estrogen treatment is similarly beneficial. Diethylstilbestrol from estrogen is another useful hormonal therapy which has a disadvantage of causing cardiovascular toxicity. When either LHRH agonists, such as leuprolide, buserelin, or goserelin, or gonadotropin-releasing hormone antagonists, such as Abarelix, are administered testosterone concentrations are ultimately reduced. Flutamide and other nonsteroidal, anti-androgen agents block binding of testosterone to its intracellular receptors. As a result, it blocks the effect of testosterone, increasing serum testosterone concentrations and allows patients to remain potent - a significant problem after radical prostatectomy and radiation treatments.

[0009] In view of the shortcoming of existing therapies, there exists a need for improved modalities for preventing and treating cancers, such as prostate cancer.

SUMMARY OF THE INVENTION

[0010] This invention provides, *inter alia*, antibodies and particularly, modified antibodies, or antigen-binding fragments thereof, that bind to the extracellular domain of human prostate specific membrane antigen (PSMA). The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. The modified anti-PSMA antibodies, or fragments thereof, bind to human PSMA with high affinity and specificity, and thus can be used as diagnostic, prophylactic, or therapeutic agents *in vivo* and *in vitro*. Accordingly, the invention provides antibodies and particularly modified anti-PSMA antibodies, antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect PSMA, or to ablate or kill a PSMA-expressing cell, e.g., a PSMA-expressing cancer, a prostatic, or a vascular cell, either *in vitro* or *in vivo*, are also encompassed by the invention. Preferably, the modified antibodies are those having one or more complementarity determining regions (CDRs) from a J591, J415, J533 or E99 antibody. As discussed herein, the modified antibodies can be CDR-grafted, humanized, deimmunized, or, more generally, antibodies having the CDRs from a non-human antibody, e.g., murine J591, J415, J533 or E99

antibody, and a framework that is selected as less immunogenic in humans, e.g., less antigenic than the murine frameworks in which a murine CDR naturally occurs.

[0011] The antibodies, e.g., modified antibodies of the invention interact with, e.g., bind to, PSMA, preferably human PSMA, with high affinity and specificity. For example, the antibody binds to human PSMA with an affinity constant of at least 10^7 M^{-1} , preferably between 10^8 M^{-1} and 10^{10} M^{-1} , or about 10^9 M^{-1} . Preferably, the antibody interacts with, e.g., binds to, the extracellular domain of PSMA, and most preferably, the extracellular domain of human PSMA (e.g., amino acids 44-750 of human PSMA).

[0012] In some embodiments, the anti-PSMA antibody binds all or part of an epitope bound by an antibody described herein, e.g., a J591, E99, J415, and J533 antibody. The anti-PSMA antibody can inhibit, e.g., competitively inhibit, the binding of an antibody described herein, e.g., a J591, E99, J415, and J533 antibody, to human PSMA. An anti-PSMA antibody may bind to an epitope, e.g., a conformational or a linear epitope, which epitope when bound prevents binding of an antibody described herein, e.g., a J591, E99, J415, and J533 antibody. The epitope can be in close proximity spatially or functionally-associated, e.g., an overlapping or adjacent epitope in linear sequence or conformational space, to the one recognized by the J591, E99, J415, or J533 antibody.

[0013] In some embodiments, the anti-PSMA antibody binds to an epitope located wholly or partially within the region of about amino acids 120 to 500, preferably 130 to 450, more preferably, 134 to 437, or 153 to 347, of human PSMA. Preferably, the epitope includes at least one glycosylation site, e.g., at least one N-linked glycosylation site (e.g., the asparagine residue located at about amino acids 190-200, preferably at about amino acid 195, of human PSMA).

[0014] Human PSMA is expressed on the surface of normal, benign hyperplastic, and cancerous prostate epithelial cells, as well as vascular endothelial cells proximate to cancerous cells, e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells. The expression of human PSMA is substantially lower on non-malignant prostate cells where PSM', a splice variant that lacks a portion of the N-terminal domain that includes the transmembrane domain, is more abundant. Due to the absence of the N-terminal region

containing the transmembrane domain, PSM' is primarily cytoplasmic and is not located on the cell membrane. The antibodies, e.g., the modified antibodies, of the invention bind to the cell surface of cells that express PSMA. PSMA is normally recycled from the cell membrane into the cell. Thus, the antibodies of the invention are internalized with PSMA through the process of PSMA recirculation, thereby permitting delivery of an agent conjugated to the antibody, e.g., a labeling agent, a cytotoxic agent, or a viral particle (e.g., a viral particle containing genes that encode cytotoxic agents, e.g., apoptosis-promoting factors). Accordingly, antibodies, e.g., modified antibodies, described herein, can be used to target living normal, benign hyperplastic, and cancerous prostate epithelial cells, as well as vascular endothelial cells proximate to cancerous cells.

[0015] An antibody, e.g., a modified antibody, is preferably monospecific, e.g., a monoclonal antibody, or an antigen-binding fragment thereof. The antibodies, e.g., the modified antibodies, can be full-length (e.g., an IgG (e.g., an IgG1, IgG2, IgG3, IgG4), IgM, IgA (e.g., IgA1, IgA2), IgD, and IgE, but preferably an IgG) or can include only an antigen-binding fragment (e.g., a Fab, F(ab')₂ or scFv fragment, or one or more CDRs). An antibody, or antigen-binding fragment thereof, can include two heavy chain immunoglobulins and two light chain immunoglobulins, or can be a single chain antibody. The antibodies can, optionally, include a constant region chosen from a kappa, lambda, alpha, gamma, delta, epsilon or a mu constant region gene. A preferred anti-PSMA antibody includes a heavy and light chain constant region substantially from a human antibody, e.g., a human IgG1 constant region, a portion thereof, or a consensus sequence.

[0016] In a preferred embodiment, the antibodies (or fragments thereof) are recombinant or modified anti-PSMA antibodies chosen from, e.g., a chimeric, a humanized, a deimmunized, or an *in vitro* generated antibody. In other embodiments, the anti-PSMA antibodies are human antibodies. In one embodiment, a modified antibody of the invention is a deimmunized anti-PSMA antibody, e.g., a deimmunized form of E99, J415, J533 or J591 (e.g., a deimmunized form of an antibody produced by a hybridoma cell line having an ATCC Accession Number HB-12101, HB-12109, HB-12127, and HB-12126, respectively). Preferably, a modified antibody is a deimmunized form of J591 or J415 (referred to herein as "deJ591" or "deJ415", respectively). Most preferably, the antibody is a deimmunized form of J591.

[0017] Any combination of anti-PSMA antibodies is within the scope of the invention, e.g., two or more antibodies that bind to different regions of PSMA, e.g., antibodies that bind to two different epitopes on the extracellular domain of PSMA.

[0018] In some embodiments, the anti-PSMA antibody, e.g., the modified anti-PSMA antibody or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin (or preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin). Preferably, each immunoglobulin includes a light or a heavy chain variable region having at least one, two and, preferably, three CDRs substantially identical to a CDR from a non-human anti-PSMA light or heavy chain variable region, respectively. For example, the antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from: the heavy chain variable region of murine J591 (see SEQ ID NO:1, 2, and 3, depicted in Figure 1A); the light chain variable region of murine J591 (see SEQ ID NO:4, 5, and 6, depicted in Figure 1B); the heavy chain variable region of murine J415 (see SEQ ID NO:29, 30, and 31, depicted in Figure 5); the light chain variable region of murine J415 (see SEQ ID NO:32, 33, and 34, depicted in Figure 6); the heavy chain variable region of murine J533 (see SEQ ID NO:93, 94, and 95, depicted in Figure 9A); the light chain variable region of murine J533 (see SEQ ID NO:96, 97, and 98, depicted in Figure 10A); the heavy chain variable region of murine E99 (see SEQ ID NO:99, 100, and 101, depicted in Figure 11A); or the light chain variable region of murine E99 (see SEQ ID NO:102, 103, and 104, depicted in Figure 12A). In other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two, and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12126 or the deimmunized J591 antibody produced by the cell line having ATCC Accession Number PTA-3709. In other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12109 or the deimmunized J415 antibody produced by a cell line having ATCC Accession Number PTA-4174. In still other embodiments, the modified antibody or antigen-binding fragment thereof can have at least one, two and preferably three CDRs from the light or heavy chain variable region of the antibody produced by the cell line having ATCC Accession Number HB-12127 or the antibody produced by a cell line having ATCC Accession Number HB-12101.

[0019] In one preferred embodiment, the modified antibody or antigen-binding fragment thereof includes all six CDRs from the same non-human anti-PSMA antibody, e.g., a murine J591, J415, J533 or E99 antibody. In some embodiments, the CDRs have the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5 and 6 (corresponding to murine J591 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession number HB-12126, or the deimmunized J591 antibody produced by the cell line having ATCC Accession Number PTA-3709, or sequences substantially identical thereto. In other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:29, 30, 31, 32, 33, and 34 (corresponding to murine J415 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12109, or the deimmunized J415 antibody produced by the cell line having ATCC Accession Number PTA-4174, or sequences substantially identical thereto. In other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:93, 94, 95, 96, 97, and 98 (corresponding to murine J533 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12127, or sequences substantially identical thereto. In still other embodiments, the CDRs have the amino acid sequences of SEQ ID NO:99, 100, 101, 102, 103, and 104 (corresponding to murine E99 heavy and light chain CDRs), the amino acid sequences of the CDRs of the antibody produced by the cell line having ATCC Accession Number HB-12101, or sequences substantially identical thereto.

[0020] The amino acid sequence of the CDRs for antibodies J591, J415, J533 and E99 are provided below in Table 1.

Table 1: CDR Sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V _H CDR1 J591	Mus musculus	Fig. 1A	1	GYTFTEYTIH
V _H CDR2 J591	Mus musculus	Fig. 1A	2	NINPNNGGTTYNQKFED
V _H CDR3 J591	Mus musculus	Fig. 1A	3	GWNFDY
V _L CDR1	Mus musculus	Fig. 1B	4	KASQDVGTAVD

J591				
V _L CDR2 J591	Mus musculus	Fig. 1B	5	WASTRHT
V _L CDR3 J591	Mus musculus	Fig. 1B	6	QQYNSYPLT
V _H CDR1 J415	Mus musculus	Fig. 5	29	GFIFSNYWMN
V _H CDR2 J415	Mus musculus	Fig. 5	30	EIRSQSNNFATHYAESVKG
V _H CDR3 J415	Mus musculus	Fig. 5	31	RWNNF
V _L CDR1 J415	Mus musculus	Fig. 6	32	KASENVGTYVS
V _L CDR2 J415	Mus musculus	Fig. 6	33	GASNRFT
V _L CDR3 J415	Mus musculus	Fig. 6	34	GQSYTFPYT
V _H CDR1 J533	Mus musculus	Fig. 9A	93	GYTFTGYVMH
V _H CDR2 J533	Mus musculus	Fig. 9A	94	YINPYNDVTRYNGKFKG
V _H CDR3 J533	Mus musculus	Fig. 9A	95	GENWYYFDS
V _L CDR1 J533	Mus musculus	Fig. 10A	96	RASEIDSYDNTFMH
V _L CDR2 J533	Mus musculus	Fig. 10A	97	RASILES
V _L CDR3 J533	Mus musculus	Fig. 10A	98	HQSIEDPYT
V _H CDR1 E99	Mus musculus	Fig. 11A	99	GFSLTAYGIN
V _H CDR2 E99	Mus musculus	Fig. 11A	100	VIWPDGNTDYNSTLKS
V _H CDR3 E99	Mus musculus	Fig. 11A	101	DSYGNFKRGWFDF
V _L CDR1 E99	Mus musculus	Fig. 12A	102	KASQNVGSDVA
V _L CDR2 E99	Mus musculus	Fig. 12A	103	STSYRYS
V _L CDR3 E99	Mus musculus	Fig. 12A	104	QQYNSYPLT

[0021] The light or heavy chain immunoglobulin of the modified anti-PSMA antibody or antigen-binding fragment thereof can further include a light chain or a heavy chain variable framework sequence from a light chain or heavy chain variable framework present in a human or a non-human, e.g., rodent, antibody (e.g., the murine J591, J415, J533 or E99 antibody heavy

chain or light chain variable framework). In some embodiments, the light chain or the heavy chain variable framework can be chosen from:

- i a light or heavy chain variable framework including at least 5, 10, 20, 30, 40, 50, 60, 70, or 80 amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a human consensus antibody sequence;
- ii a light or heavy chain variable framework including at least 5, but less than 30, amino acid residues from a human light chain or heavy chain variable framework, e.g., a light chain or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a human consensus antibody sequence;
- iii a light or heavy chain variable framework including at least 5, 10, 20, 30, 40, 50, 60, 75 or more amino acid residues from a light or heavy variable framework from a non-human antibody, e.g., a murine antibody (e.g., an anti-PSMA antibody having the framework amino acid sequence shown in SEQ ID NO:7 or 8 (from the heavy and light chain, respectively, of murine J591; see Figures 1A and 1B), SEQ ID NO:35 or 36 (from the heavy and light chain, respectively, of murine J415; see Figures 5 and 6), SEQ ID NO:109 or 114 (from the heavy and light chain, respectively, of murine J533; see Figures 9A and 10A), or SEQ ID NO:119 or 124 (from the heavy and light chain, respectively, of murine E99; see Figures 11A and 12A), or the framework of a murine antibody described herein (e.g., a murine J591, J415, J533, or E99 antibody produced by a hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101);
- iv a light or heavy chain variable framework, which has at least 60%, 65%, 70%, 72%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identity with, or which has an amino acid sequence which differs by at least 1, 2, 5, or more residues, but less than 10, 20, 30 or 40 residues from, the sequence of the framework of a light or heavy chain variable region of a non-human antibody, e.g., a murine antibody (e.g., an anti-PSMA antibody having the framework amino acid sequence shown in SEQ ID NO:7 or 8 (from the heavy and light chain, respectively, of murine J591; see Figures 1A and 1B), SEQ ID NO:35 or 36 (from the heavy and light chain, respectively, of murine J415; see Figures 5 and 6), SEQ ID NO:109 or 114 (from the heavy and light chain, respectively,

cell line having ATCC Accession Number PTA-3709 or PTA-4174. Preferably, the heavy or light chain framework region includes the amino acid sequence shown in SEQ ID NO:17 or SEQ ID NO:18, respectively, SEQ ID NO:45 or SEQ ID NO:46, respectively, or the heavy or light chain framework sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0023] In other embodiments, the heavy or light chain variable region of the modified anti-PSMA antibody has an amino acid sequence which has at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or more identity with SEQ ID NO:21 or SEQ ID NO:22, respectively (corresponding to the heavy and light chain variable regions of deimmunized J591; see Figures 2A-2B), SEQ ID NO:49 or SEQ ID NO:50, respectively (corresponding to the heavy and light chain variable regions of deimmunized J415, J415DIVH4 and J415DIVK5; see Figures 5 or 6), or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174. In other embodiments, the heavy or light chain variable region of the modified anti-PSMA antibody has an amino acid sequence that differs by at least 1, 2, 5, or more residues, but less than 10, 20, 30, or 40 residues, from the amino acid sequence of SEQ ID NO:21 or SEQ ID NO:22, respectively, SEQ ID NO:49 or SEQ ID NO:50, respectively, or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174. Preferably, the light or heavy chain variable region includes the amino acid sequence shown in SEQ ID NO:21 or SEQ ID NO:22, respectively, SEQ ID NO:49 or SEQ ID NO:50, respectively, or the heavy or light chain variable region sequence of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0024] Preferred modified anti-PSMA antibodies include at least one, preferably two, light chain variable regions and at least one, preferably two, heavy chain variable regions having the amino acid sequence shown in SEQ ID NO:21 and SEQ ID NO:22, respectively (corresponding to the heavy and light chain variable regions of deimmunized J591; see Figures 2A-2B), SEQ ID NO:49 and SEQ ID NO:50, respectively (corresponding to the heavy and light chain variable regions of deimmunized J415, J415DIVH4 and J415DIVK5; see Figures 5 and 6), or at least one, preferably two, modified light chain variable region sequences and at least one, preferably two, heavy chain variable region sequences of the antibody produced by the cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[0025] In other embodiments, the light or heavy chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one, two, three, four, five, six, seven, eight, nine, ten, fifteen, sixteen, or seventeen amino acid residues from a human light or heavy chain variable framework, e.g., a light or heavy chain variable framework residue from a mature human antibody, a human germline antibody sequence, or a consensus antibody sequence.

[0026] In some embodiments, the amino acid residue from the human light chain variable framework is the same as the residue found at the same position in a human germline antibody sequence. Preferably, the amino acid residue from the human light chain variable framework is the most common residue at the same position in the human germline antibody sequence. Preferably, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, ten amino acid residues which differ from the framework of the non-human anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region), or which is from a human light chain variable framework (e.g., a human germline, mature, or consensus framework sequence), at a position selected from the group consisting of: residue 8, 9, 10, 11, 20, 22, 60, 63, 76, 77, 78, 80, 83, 87, 103, 104 and 106 (Kabat numbering as shown in Table 2). Preferably, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, or ten amino acid residues from the human light chain variable framework selected from the group consisting of: residue 8 (proline), 9 (serine), 10 (serine), 11 (leucine), 20 (threonine), 22 (threonine), 60 (serine), 63 (serine), 76 (serine), 77 (serine), 78 (leucine), 80 (proline), 83 (phenylalanine), 87 (tyrosine), 103 (lysine), 104 (valine) and 106 (isoleucine) (Kabat numbering as shown in Table 2).

[0027] The amino acid replacements in the deimmunized J591 light chain variable region are provided below in Table 2. The left panel indicates the amino acid number according to Kabat, E.A., *et al.* (1991) *supra*; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding position in the human germline.

Table 2

Position Kabat No	Substitution of mouse sequence	Most common in human germline
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3	V→Q	V
8	H→P	P
9	K→S	S
10	F→S	S
11	M→L	L
20	S→T	T
21	I→L	I
22	I→T	T
42	Q→P	K
58	V→I	V
60	D→S	S
63	T→S	S
76	T→S	S
77	T→S	S
78	V→L	L
80	S→P	P
83	L→F	F
87	F→Y	Y
100	A→P	Q
103	M→K	K
104	L→V	V
106	L→I	I

[0028] In other embodiments, the light chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, or seven amino acid residues which differ from the framework of a non-human anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region), or which is from a human light chain variable framework (e.g., a human germline, mature, or consensus framework), at a position selected from the group consisting of: residue 13, 15, 19, 41, 63, 68, and 80 (linear numbering as shown in Figure 6). Preferably, the light chain variable framework of the modified antibody, or antigen-binding fragment thereof, has at least one, two, three, five, or seven amino acid residues from the human consensus light chain variable framework selected from the group consisting of: residue 13 (alanine), 15 (alanine), 19 (methionine), 41 (threonine), 63 (serine), 68 (glycine), and 80 (alanine) (linear numbering as shown in Figure 6).

[0029] The amino acid replacements in the deimmunized J415 light chain variable region are provided below in Table 3. The left panel indicates the amino acid number using linear numbering; the middle panel indicates the replacements of the residue in the mouse sequence and

the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding position in the human germline.

Table 3

Position Linear No	Substitution of mouse sequence	Most common in human germline
13	I→A	A
15	V→A	A
19	V→M	M
41	E→T	T
63	T→S	S
68	A→G	G
80	T→A	A

[0030] In other embodiments, the light chain variable framework of the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least 5, but no more than 80, amino acid residues from the light chain variable framework shown in SEQ ID NO:8 (from murine J591; see Figure 1B), SEQ ID NO:36 (from murine J415; see Figure 6), SEQ ID NO:114 (from murine J533; see Figure 10A), or SEQ ID NO:124 (from murine E99; see Figure 12A), or the light chain variable framework of an antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101. Preferably, the light chain variable framework has at least 60%, 65%, 70%, 72%, 75%, 80%, 85%, 90%, or 94% identity with, or differs by at least 5, 7, 10, 20, or 30 but less than 10, 20, 30, or 40 amino acid residues from, the non-human light chain variable framework, e.g., the murine J591 or J415 light chain variable framework shown in SEQ ID NO:8 or SEQ ID NO:36, respectively, or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109. In other embodiments, the light chain variable framework is from murine J591 antibody (SEQ ID NO:8; see Figure 1B), from murine J415 antibody (SEQ ID NO:36; see Figure 6), from murine J533 antibody (SEQ ID NO:114; see Figure 10A), or from murine E99 antibody (SEQ ID NO:124; see Figure 12A), or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101.

[0031] In yet other embodiments, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine)

light chain variable framework (e.g., a murine J591 light chain variable framework as shown in SEQ ID NO:8 or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) which has at least 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, or 23 amino acid replacements. In one embodiment, the non-human light chain variable framework includes one or more of:

- a framework region 1 having at least 5, 6, 7, or 8 replacements;
- a framework region 2 having at least one replacement;
- a framework region 3 having at least 5, 6, 7, 8, or 9 replacements; or
- a framework region 4 having at least 2, 3 or 4 replacements.

[0032] In yet other embodiments, the light chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) light chain variable framework (e.g., a murine J415 light chain variable framework as shown in SEQ ID NO:36 or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) which has at least 1, 2, 3, 4, 5, 6, 7, 8, or 10 amino acid replacements. In some embodiments, the non-human light chain variable framework includes one or more of:

- a framework region 1 having at least 1, 2 or 3 replacements;
- a framework region 2 having at least one replacement; or
- a framework region 3 having at least 1, 2 or 3 replacements.

[0033] The replacement can be selected from: a conservative substitution of a non-human residue, or a residue found in a human germline, mature or consensus framework sequence at the same position, e.g. the most common residue in the human germline sequence at the same position. In some embodiments, the light chain variable framework has at least 3, 4 and preferably 5 conservative substitutions. In other embodiments, the light chain variable framework has at least 5, 7, 10, 15, 16, or 17 amino acid replacements wherein the replacement amino acid residue is the most common residue in the human germline framework sequence at the same position.

[0034] In some embodiments, the non-human light chain variable framework has at least one, two, three, five, seven, ten, eleven, fifteen, sixteen, seventeen, nineteen, twenty, twenty-one or twenty-two amino acid replacements at a position selected from the group consisting of: residue 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, 104 and 106

(Kabat numbering as shown in Table 2). The replacement can be chosen from one or more of: residue 3 (glutamine), 8 (proline), 9 (serine), 10 (serine), 11 (leucine), 20 (threonine), 21 (leucine), 22 (threonine), 42 (proline), 58 (isoleucine), 60 (serine), 63 (serine), 76 (serine), 77 (serine), 78 (leucine), 80 (proline), 83 (phenylalanine), 87 (tyrosine), 100 (proline), 103 (lysine), 104 (valine) and 106 (isoleucine) (Kabat numbering as shown in Table 2).

[0035] In other embodiments, the non-human light chain variable framework has at least one, two, three, five, or seven amino acid replacements at a position selected from the group consisting of: residue 13, 15, 19, 41, 63, 68 and 80 (linear numbering as shown in Table 3). Preferably, the light chain variable framework of the modified antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven amino acid residues from the human consensus light chain variable framework selected from the group consisting of: residue 13 (alanine), 15 (alanine), 19 (methionine), 41 (threonine), 63 (serine), 68 (glycine) and 80 (alanine) (linear numbering as shown in Table 3).

[0036] Preferably, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, five, seven, or eight amino acid residues, which differ from the framework of the non-human anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region), or which is from a human heavy chain variable framework (e.g., a human germline framework), at a position selected from the group consisting of: residue 5, 40, 41, 44, 82a, 83, 87, and 108 (Kabat numbering as shown in Table 4). Preferably, the heavy chain variable framework of the recombinant antibody, or antigen-binding fragment thereof, has at least one amino acid residue from the human heavy chain variable framework selected from the group consisting of: residue 5 (valine), 40 (alanine), 41 (proline), 44 (glycine), 82a (serine), 83 (arginine), 87 (threonine), or 108 (leucine) (Kabat numbering as shown in Table 4).

[0037] The amino acid replacements in the deimmunized J591 heavy chain variable region are provided below in Table 4. The left panel indicates the amino acid number according to Kabat, E.A., et al. (1991) supra; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding position in the human germline.

Table 4

Position Kabat No.	Substitution of mouse sequence	Most common in human germline
5	Q→V	V
11	L→V	L
12	V→K	V
16	T→A	G
17	S→T	S
19	R→K	R
40	S→A	A
41	H→P	P
44	S→G	G
75	S→T	K
76	S→D	N
82a	R→S	S
83	T→R	R
87	S→T	T
108	T→L	L

[0038] In other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, has at least one, two, three, four, five amino acid residues, which differ from the framework of a non-human anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region), or which is from a human heavy chain variable framework (e.g., a human mature, consensus, or germline framework), at a position selected from the group consisting of: residue 20, 87, 94, 95, and 112 (linear numbering as shown in Table 5). Preferably, the heavy chain variable framework of the recombinant antibody, or antigen-binding fragment thereof, has at least one, two, three, four, five amino acid residues from the human heavy chain variable framework selected from the group consisting of: residue 20 (isoleucine), 87 (serine), 94 (alanine), 95 (valine), and 112 (valine) (linear numbering as shown in Table 5).

[0039] The amino acid replacements in the deimmunized J415 heavy chain variable region are provided below in Table 5. The left panel indicates the linear amino acid number; the middle panel indicates the replacements of the residue in the mouse sequence and the corresponding mouse residues; and the right panel indicates the most common residue in the corresponding position in the human germline.

Table 5

Position	Substitution	Most common in
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Kabat No	of mouse sequence	human germline
20	L→I	I
87	N→S	S
94	G→A	A
95	I→V	V
112	L→V	V

[0040] In other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes at least 5 but no more than 75 or 82 amino acid residues from the heavy chain variable framework shown in SEQ ID NO:7 (from murine J591; see Figure 1A), SEQ ID NO:35 (from murine J415; see Figure 5), SEQ ID NO:109 (from murine J533; see Figure 9A), or SEQ ID NO:119 (from murine E99; see Figure 11A), or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101. Preferably, the heavy chain variable framework has at least 60%, 65%, 70%, 80%, 82%, 85%, 90%, or 94% identity with, or differs by at least 5, 10, 20, or 30 but less than 10, 20, 30, or 40 residues from, a non-human heavy chain variable framework, e.g., the murine J591 or J415 or heavy chain variable framework shown in SEQ ID NO:7 or SEQ ID NO:35, respectively, or a heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109. In other embodiments, the non-human heavy chain variable framework is from murine J591 antibody (SEQ ID NO:7; see Figure 1A), from murine J415 antibody (SEQ ID NO:35; see Figure 5), from murine J533 antibody (SEQ ID NO:109; see Figure 9A), or from murine E99 antibody (SEQ ID NO:119; see Figure 11A), or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126, HB-12109, HB-12127 or HB-12101.

[0041] In yet other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) heavy chain variable framework (e.g., a murine J591 heavy chain variable framework (SEQ ID NO:7, as shown Figure 1A, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) which has at least 3, 5, 10, 15, 16, 17, 18, or 19 amino acid replacements. In one embodiment, the non-human heavy chain variable framework of the modified anti-PSMA antibody includes one or more of:

a framework region 1 having at least 4, 5, or 6 replacements;

- a framework region 2 having at least 1, 2, or 3 replacements;
- a framework region 3 having at least 3, 4, or 5 replacements; or
- a framework region 4 having at least one replacement.

[0042] In yet other embodiments, the heavy chain variable framework of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human (e.g., a murine) heavy chain variable framework (e.g., a murine J415 heavy chain variable framework (SEQ ID NO:35, as shown in Figure 5, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) which has at least 1, 2, 3, 4, or 5 amino acid replacements. In one embodiment, the non-human heavy chain variable framework of the modified anti-PSMA antibody includes one or more of:

- a framework region 1 having at least one replacement;
- a framework region 3 having at least 1, 2, or 3 replacements; or
- a framework region 4 having at least one replacement.

[0043] The replacement can be chosen from: a conservative substitution of a non-human residue, or a residue found in a human germline, mature or consensus sequence at the same position, e.g. the most common residue in the human germline at the same position. In one embodiment, the heavy chain variable framework has at least 3, 4, 5, 6 and preferably 7 conservative substitutions. Preferably, the heavy chain variable framework has at least 5, 6, 7 and preferably 8 replacements by the most common residue in the human germline at the same position.

[0044] In some embodiments, the non-human heavy chain variable framework has at least one amino acid replacement at a position selected from the group consisting of: residue 5, 11, 12, 16, 17, 19, 40, 41, 44, 75, 76, 82a, 83, 87, and 108 (Kabat numbering as shown in Table 3). The replacement can be chosen from one or more of: 5 (valine), 11 (valine), 12 (lysine), 16 (alanine), 17 (threonine), 19 (lysine), 40 (alanine), 41 (proline), 44 (glycine), 75 (threonine), 76 (aspartate), 82a (serine), 83 (arginine), 87 (threonine), and 108 (leucine) (Kabat numbering as shown in Table 4).

[0045] In other embodiments, the non-human heavy chain variable framework has at least one amino acid replacement at a position selected from the group consisting of: residue 20, 87, 94, 95 and 112 (linear numbering as shown in Table 5). The replacement can be chosen from

one or more of: residue 20 (isoleucine), 87 (serine), 94 (alanine), 95 (valine), and 112 (valine) (linear numbering as shown in Table 5).

[0046] The amino acid sequence of the framework regions of the light and heavy chains regions of antibodies J591, J415, J533 and E99 are provided in Table 6, below.

Table 6: Framework Sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V _H FR1-FR4 J591	Mus musculus	Fig. 1A	7	EVQLQQSGPELKKPGTSVRISCK TSWVKQSHGKSLEWIGKATLTV DKSSSTAYMELRSLTSEDSAVY YCAAWGQGTTTLTVSS
V _L FR1-FR4 J591	Mus musculus	Fig. 1B	8	DIVMTQSHKFMSTSVGDRVSIIC WYQQKPGQSPKLLIYGVPDRFT GSGSGTDFLTITNVQSEDLADY FCFGAGTMLDLK
V _H FR1 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	9	EVQLVQSGPEVKKPGATVKISC KTS
V _H FR2 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	10	WVKQAPGKGLEWIG
V _H FR3 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	11	KATLTVDKSTD TAYMELSSLRS EDTAVYYCAA
V _H FR4 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	12	WGQGTLTLTVSS
V _L FR1 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	13	DIQMTQSPSSLSTSVGDRVTLTC
V _L FR2 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	14	WYQQKPGPSPKLLIY
V _L FR3 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	15	GIPSRFSGSGSGTDFLTISLQPE DFADYYC

V _L FR4 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	16	FGPGTKVDIK
V _H FR1-FR4 (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	17	EVQLVQSGPEVKKPGATVKISC KTSWVKQAPGKGLEWIGKATLT VDKSTDYAMELSSLRSEDYAV YYCAAWGQGTLTVSS
V _L FR1-FR4 (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	18	DIQMTQSPSSLSTSVGDRVTLTC WYQQKPGSPKLLIYGIPSRFSGS GSGTDFLTITSSLPEDFADYYC FGPGTKVDIK
V _H FR1-FR4 J415	Mus musculus	Fig. 5	35	EVKLEESGGGLVQPGGSMKLS VASWVRQSPEKGLEWVARVIS RDDSKSSVYLQMNRLAEDTGI YYCTRWGQGTLTVSS
V _L FR1-FR4 J415	Mus musculus	Fig. 6	36	NIVMTQFPKSMISVGERVTLTC WYQQKPEQSPKMLIYGVPDRFT GSGSATDFLTITSSVQTEDLVY YCFGGGKLEMK
V _H FR1 (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	37	EVKLEESGGGLVQPGGSMKISC VAS
V _H FR2 (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	38	WVRQSPEKGLEWVA
V _H FR3 (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	39	RVISRDDSKSSVYLQMNRLAE DTAVYYCTR
V _H FR4 (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	40	WGQGTTVTVSS
V _L FR1 (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	41	NIVMTQFPKSMISASAGERMTLT C
V _L FR2 (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	42	WYQQKPTQSPKMLIY
V _L FR3 (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	43	GVPDRFSGSGGTDFLTITSSVQA EDLVYYC

V _L FR4 (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	44	FGGGTKLEMK
V _H FR1-FR4 (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	45	EVKLEESGGGLVQPGGSMKISC VASWVRQSPEKGLEWVARVIIS RDDSKSSVYLQMNSLRAEDTAV YYCTRWGQGTTVTVSS
V _L FR1-FR4 (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	46	NIVMTQFPKSMSASAGERMTLT CWYQQKPTQSPKMLIYGVPDRF SGSGSGTDFILTISVQAEDLV DY YCFGGGTKLEMK
V _H FR1 J533	Mus musculus	Fig. 9A	105	EVQLQQSGPELVKPGASVKMSC KAS
V _H FR2 J533	Mus musculus	Fig. 9A	106	WVKQKPGQVLEWIG
V _H FR3 J533	Mus musculus	Fig. 9A	107	KATLTSDKYSSTAYMELSGLTSE DSAVYYCAR
V _H FR4 J533	Mus musculus	Fig. 9A	108	WGRGATLTVSS
V _H FR1-FR4 J533	Mus musculus	Fig. 9A	109	EVQLQQSGPELVKPGASVKMSC KASWVKQKPGQVLEWIGKATLT SDKYSSTAYMELSGLTSEDSAV YYCARWGRGATLTVSS
V _L FR1 J533	Mus musculus	Fig. 10A	110	DIVLTQSPASLAVSLGQRATISC
V _L FR2 J533	Mus musculus	Fig. 10A	111	WYQQKPGQPPNLLIF
V _L FR3 J533	Mus musculus	Fig. 10A	112	GIPARFSGSGSGTDFLTITYPVEA DDVATYYC
V _L FR4 J533	Mus musculus	Fig. 10A	113	FGGGTKLEIK

V _L FR1-FR4 J533	Mus musculus	Fig. 10A	114	DIVLTQSPASLAVSLGQRATISC WYQQKPGQPPNLLIFGIPARFSG SGSGTDFLTITYPVEADDVATYY CFGGGTKLEIK
V _H FR1 E99	Mus musculus	Fig. 11A	115	QVQLKESGPGGLVASSQSLTCT VS
V _H FR2 E99	Mus musculus	Fig. 11A	116	WVRQPPGKGLEWLG
V _H FR3 E99	Mus musculus	Fig. 11A	117	RLNIFKDNSKNQVFLKMSSFQTD DTARYFCAR
V _H FR4 E99	Mus musculus	Fig. 11A	118	WGQGTTLTVSS
V _H FR1-FR4 E99	Mus musculus	Fig. 11A	119	QVQLKESGPGGLVASSQSLTCT VSWVRQPPGKGLEWLGRLNIFK DNSKNQVFLKMSSFQTD DTARYFCARWGQGTTLTVSS
V _L FR1 E99	Mus musculus	Fig. 12A	120	NIVMTQSQKFMSTSPGDRVRVT C
V _L FR2 E99	Mus musculus	Fig. 12A	121	WYQAKPGQSPRLIY
V _L FR3 E99	Mus musculus	Fig. 12A	122	GVPDRFTAYGSGTDFLTITNVQ SEDLTEYFC
V _L FR4 E99	Mus musculus	Fig. 12A	123	FGAGTKLELK
V _L FR1-FR4 E99	Mus musculus	Fig. 12A	124	NIVMTQSQKFMSTSPGDRVRVT CWYQAKPGQSPRLIYGVPDRFT AYGSGTDFLTITNVQSEDLTEY FCFGAGTKLELK

[0047] In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light chain or heavy chain immunoglobulin or, preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin. Preferably, the light chain

immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 or J415 light chain variable region shown in SEQ ID NO:20 (see Figure 1B) or SEQ ID NO:48 (see Figure 6), respectively, or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109) and a light chain framework which differs from the framework of the non-human, e.g., murine, anti-PSMA light chain framework (e.g., the murine J591 or J415 light chain framework shown in SEQ ID NO:8 (see Figure 1B) or SEQ ID NO:36 (see Figure 6), respectively, or the light chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or 12109) at one, two, three, four, five, six, seven or more positions selected from the group consisting of: residue 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, 104 and 106 (Kabat numbering as in Table 2), or residues 13, 15, 19, 41, 63, 68, and 80 (linear numbering as in Table 3).

[0048] In other preferred embodiments, the heavy chain immunoglobulin includes a non-human heavy chain variable region comprising three complementarity determining regions (CDRs) from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 or J415 heavy chain variable region shown in SEQ ID NO:19 (see Figure 1A) or SEQ ID NO:47 (see Figure 5), respectively, or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109) and a modified heavy chain framework which differs from the framework of the non-human, e.g., murine, anti-PSMA heavy chain framework (e.g., the murine J591 or J415 heavy chain framework shown in SEQ ID NO:7 (see Figure 1A) or SEQ ID NO:35 (see Figure 5), respectively, or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126 or HB-12109) at one, two, three, four, five or more positions selected from the group consisting of: residue 5, 11, 12, 16, 17, 19, 40, 41, 44, 75, 76, 82a, 83, 87, and 108 (Kabat numbering as in Table 4), or residue 20, 87, 94, 95 and 112 (linear numbering as in Table 5).

[0049] In yet other embodiments, the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin or, more preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin. Preferably, the light chain immunoglobulin includes a modified non-human light chain variable region

comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region shown in SEQ ID NO:20 (see Figure 1B), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a modified light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., the murine J591 light chain variable region (SEQ ID NO:20 or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126), by at least one, two, three, four, five, six, seven, eight, nine, ten positions selected from the group consisting of:

a position within or adjacent to one or more of residues 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, or a T cell epitope which includes one or more of residues 1-13 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or a T cell epitope which includes one or more of residues residues 8-20 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29, or a T cell epitope which includes one or more of residues 17-29 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39, or a T cell epitope which includes one or more of residues 27-39 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43, or a T cell epitope which includes one or more of residues 30-43 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, or 57, or a T cell epitope which includes one or more of residues 45- 57 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, or 68, or a T cell epitope which includes one or more of residues 56-68 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, or 83, or a T cell epitope which includes one or more of residues 71- 83 (numbering as in Figure 3B);

a position within or adjacent to one or more of residues 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 or 85, or a T cell epitope which includes one or more of residues 73-85 (numbering as in Figure 3B); and

a position within or adjacent to one or more of residues 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, or 106, or a T cell epitope which includes one or more of residues 94-106 (numbering as in Figure 3B).

[0050] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin or, more preferably, at least one light chain immunoglobulin and at least one modified heavy chain immunoglobulin. Preferably, the light chain immunoglobulin includes a modified non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region shown in SEQ ID NO:48 (Figure 37), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., the murine J415 light chain variable region (SEQ ID NO:48 or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109), by at least one, two, three, four, five, six, seven positions selected from the group consisting of:

a position within or adjacent to one or more of residues 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18, or a T cell epitope which includes one or more of residues 5-18 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, or a T cell epitope which includes one or more of residues 11-24 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26, or a T cell epitope which includes one or more of residues 13-26 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, or a T cell epitope which includes one or more of residues 17-30 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or a T cell epitope which includes one or more of residues 27-40 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44, or a T cell epitope which includes one or more of residues 31-44 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, or 69, or a T cell epitope which includes one or more of residues 56-69 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, or 73, or a T cell epitope which includes one or more of residues 60-73 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, or 83, or a T cell epitope which includes one or more of residues 70-83 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83 or 84, or a T cell epitope which includes one or more of residues 71-84 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 or 86, or a T cell epitope which includes one or more of residues 73-86 (linear numbering as in Figure 6);

a position within or adjacent to one or more of residues 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, or 92, or a T cell epitope which includes one or more of residues 76-92 (linear numbering as in Figure 6); and

a position within or adjacent to one or more of residues 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, or a T cell epitope which includes one or more of residues 81-94 (linear numbering as in Figure 6).

[0051] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region shown in SEQ ID NO:19 (see Figure 1A), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region of SEQ ID NO:19 or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126), by at least one, two, three, five, seven, ten positions selected from the group consisting of:

a position within or adjacent to one or more of residues 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14, or a T cell epitope which includes one or more of residues 2-14 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22, or a T cell epitope which includes one or more of residues 10-22 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28, or a T cell epitope which includes one or more of residues 16-28 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, or 42, or a T cell epitope which includes one or more of residues 30-42 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 44, or a T cell epitope which includes one or more of residues 32-44 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55, or a T cell epitope which includes one or more of residues 43-55 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, or 58, or a T cell epitope which includes one or more of residues 46-58 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70, or a T cell epitope which includes one or more of residues 58-70 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 or 74, or a T cell epitope which includes one or more of residues 62-74 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80 or 81, or a T cell epitope which includes one or more of residues 70-81 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93, or a T cell epitope which includes one or more of residues 81-93 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, or 96, or a T cell epitope which includes one or more of residues 84-96 (numbering as in Figure 3A);

a position within or adjacent to one or more of residues 91, 92, 93, 95, 96, 97, 98, 99, 100, 101, 102, or 103, or a T cell epitope which includes one or more of residues 91- 103 (numbering as in Figure 3A); and

a position within or adjacent to one or more of residues 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, or 112, or a T cell epitope which includes one or more of residues 100-112 (numbering as in Figure 3A).

[0052] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region shown in SEQ ID NO:47 (see Figure 5), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region, e.g., the murine J591 heavy chain variable region of SEQ ID NO:47 or the heavy chain variable framework of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109), by at least one, two, three, four, five positions selected from the group consisting of:

a position within or adjacent to one or more of residues 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23, or a T cell epitope which includes one or more of residues 10-23 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29, or a T cell epitope which includes one or more of residues 16-29 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34, or a T cell epitope which includes one or more of residues 21-34 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or a T cell epitope which includes one or more of residues 30-43 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48, or a T cell epitope which includes one or more of residues 35-48 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, or 56, or a T cell epitope which includes one or more of residues 43-56 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58 or 59, or a T cell epitope which includes one or more of residues 46-59 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, or 62, or a T cell epitope which includes one or more of residues 49-62 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, or 77, or a T cell epitope which includes one or more of residues 64-77 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, or 93, or a T cell epitope which includes one or more of residues 80-93 (numbering as in Figure 5);

a position within or adjacent to one or more of residues 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99, or a T cell epitope which includes one or more of residues 86-99 (numbering as in Figure 5); and

a position within or adjacent to one or more of residues 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, or 117, or a T cell epitope which includes one or more of residues 104-117 (numbering as in Figure 5).

[0053] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin or, more preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin. Preferably, the light chain immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J591 light chain variable region shown in SEQ ID NO:20 (Figure 1B), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a light chain framework which differs from the framework of the non-human anti-PSMA light chain variable region, e.g., murine J591 light chain variable region, by at least one position while having a residue from the non-human anti-PSMA light chain variable region at at least one, two, three, five, seven, ten, fifteen, or twenty residues selected from the group consisting of 1, 2, 4-7, 12-19, 23, 31-41, 43-49, 57, 59, 61, 62, 64-75, 79, 82, 83, 85-87, 89, 98, 99, 101, 102, 105, and 106 (numbering as in Figure 3B). The light chain framework can differ at positions chosen from one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, nineteen, twenty or more residues selected from the group consisting of 3, 8, 9, 10, 11, 20, 21, 22, 42, 58, 60, 63, 76, 77, 78, 80, 83, 87, 100, 103, and 104 (numbering as in Figure 3B).

[0054] In yet other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, includes at least one light or heavy chain immunoglobulin or, more preferably, at least one light chain immunoglobulin and at least one heavy chain immunoglobulin. Preferably, the modified light chain immunoglobulin includes a non-human light chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA light chain variable region (e.g., the murine J415 light chain variable region shown in SEQ ID NO:48 (Figure 6), or the light chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a light chain framework which differs from the framework of

the non-human anti-PSMA light chain variable region, e.g., murine J415 light chain variable region, by at least one position while having a residue from the non-human anti-PSMA light chain variable region at at least one, two, three, five, seven, ten, fifteen, or twenty residues selected from the group consisting of 1-12, 14, 16-18, 20-40, 42-62, 64-67, 69-79, and 81-107 (linear numbering as in Figure 6). The modified light chain framework can differ at at least one, two, three, four, five, six, or seven positions selected from the group consisting of 13, 15, 19, 41, 63, 68 and 80 (linear numbering as in Figure 6).

[0055] In other embodiments, the heavy chain immunoglobulin of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J591 heavy chain variable region shown in SEQ ID NO:19 (Figure 1A), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12126) and a modified heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region by at least one position while having a residue from the non-human anti-PSMA heavy chain variable region at at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen residues selected from the group consisting of 1-4, 6-10, 13-15, 18, 20-25, 36-39, 42, 43, 45-49, 67-75, 78-83, 85, 86, 88-90, 92-98, 105-109, and 111-115 (numbering as in Figure 3A). The modified heavy chain framework can differ at at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, or fourteen positions selected from the group consisting of 5, 11-12, 16-17, 19, 26-35, 40-41, 44, 50-66, 76-77, 84, 87, 91, 99-104, and 110 (numbering as in Figure 3A).

[0056] In other embodiments, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a non-human heavy chain variable region comprising three CDRs from a non-human, e.g., murine, anti-PSMA heavy chain variable region (e.g., the murine J415 heavy chain variable region shown in SEQ ID NO:47 (Figure 5), or the heavy chain variable region of the antibody produced by the hybridoma cell line having an ATCC Accession Number HB-12109) and a heavy chain framework which differs from the framework of the non-human anti-PSMA heavy chain variable region by at least one position while having a residue from the non-human anti-PSMA heavy chain variable region at at least one, two, three, four, or five residues selected from the group consisting of 1-19, 21-86, 88-93,

96-111, and 113-116 (numbering as in Figure 5). The heavy chain framework can differ at a positions selected from the group consisting of 20, 87, 94, 95 and 112 (numbering as in Figure 5).

[0057] In yet another aspect, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes a heavy chain variable region comprising at least one, two, three, four, five, six, seven, eight, nine, ten, twenty, twenty-five, thirty, thirty-five, forty, forty-five, or fifty amino acid residues chosen from one or more of the following residues and located at a position chosen from one or more of: residue 1 (glutamate), 2 (valine), 4 (leucine), 7 (serine), 8 (glycine), 11 (leucine), 14 (proline), 15 (glycine), 19 (lysine), 20 (isoleucine), 21 (serine), 22 (cysteine), 25 (serine), 26 (glycine), 28 (threonine), 29 (phenylalanine), 32 (tyrosine), 36 (tryptophan), 37 (valine), 38 (arginine/lysine), 39 (glutamine), 41 (proline), 43 (lysine), 44 (glycine), 45 (leucine), 46 (glutamate), 47 (tryptophan), 51 (isoleucine), 67 (arginine/lysine), 73 (aspartate), 75 (serine), 80 (tyrosine), 85 (serine), 86 (leucine), 87 (arginine), 89 (glutamate), 90 (aspartate), 91 (threonine), 92 (alanine), 93 (valine), 94 (tyrosine), 95 (tyrosine), 96 (cysteine), 100 (tryptophan), 101 (asparagine), 105 (tryptophan), 106 (glycine), 107 (glutamine), 108 (glycine), 109 (threonine), 112 (threonine), 113 (valine), 114 (serine), or 115 (serine) (linear numbering as shown in Figure 3A).

[0058] In one embodiment, the heavy chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes one or more of:

a framework region 1 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen amino acids selected from the group consisting of residue 1 (glutamate), 2 (valine), 4 (leucine), 7 (serine), 8 (glycine), 11 (leucine), 14 (proline), 15 (glycine), 19 (lysine), 20 (isoleucine), 21 (serine), 22 (cysteine), and 25 (serine) (linear numbering as shown in Figure 3A);

a CDR1 having at least one, two, three, four amino acids selected from the group consisting of residue 26 (glycine), 28 (threonine), 29 (phenylalanine), and 32 (tyrosine) (linear numbering as shown in Figure 3A);

a framework region 2 having at least one, two, three, four, five, six, seven, eight, nine, ten amino acids selected from the group consisting of residue 36 (tryptophan), 37 (valine), 38 (arginine/lysine), 39 (glutamine), 41 (proline), 43 (lysine), 44 (glycine), 45 (leucine), 46 (glutamate), and 47 (tryptophan) (linear numbering as shown in Figure 3A);

a CDR2 having at least one isoleucine at position 51 (linear numbering as shown in Figure 3A);

a framework region 3 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen amino acids selected from the group consisting of residue 67 (arginine/lysine), 73 (aspartate), 75 (serine), 80 (tyrosine), 85 (serine), 86 (leucine), 87 (arginine), 89 (glutamate), 90 (aspartate), 91 (threonine), 92 (alanine), 93 (valine), 94 (tyrosine), 95 (tyrosine), and 96 (cysteine) (linear numbering as shown in Figure 3A);

a CDR3 having at least one, two amino acids selected from the group consisting of residue 100 (tryptophan) and 101 (asparagine) (linear numbering as shown in Figure 3A); or

a framework region 4 having at least one, two, three, four, five, six, seven, eight, nine amino acids selected from the group consisting of residue 105 (tryptophan), 106 (glycine), 107 (glutamine), 108 (glycine), 109 (threonine), 112 (threonine), 113 (valine), 114 (serine), and 115 (serine) (linear numbering as shown in Figure 3A).

[0059] In yet another embodiment, the light chain immunoglobulin of the modified anti-PSMA antibody, or antigen-binding fragment thereof, includes a light chain variable region comprising at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, twenty, thirty, forty, fifty, sixty, or seventy amino acids chosen from one or more of the following residues and located at a position chosen from one or more of: residue 2 (isoleucine), 4 (methionine), 5 (threonine), 6 (glutamine), 8 (proline), 10 (serine), 12 (serine), 14 (serine), 16 (glycine), 17 (glutamate/aspartate), 18 (arginine), 20 (threonine), 21 (leucine), 22 (threonine), 23 (cysteine), 24 (lysine), 25 (alanine), 26 (serine), 29 (valine), 30 (glycine), 31 (threonine), 33 (valine), 35 (tryptophan), 36 (tyrosine), 37 (glutamine), 38 (glutamine), 39 (lysine), 40 (proline), 43 (serine), 44 (proline), 45 (lysine), 47 (leucine), 48 (isoleucine), 49 (tyrosine), 51 (alanine), 52 (serine), 54 (arginine), 56 (threonine), 57 (glycine), 59 (proline), 61 (arginine), 62 (phenylalanine), 63 (serine), 64 (glycine), 65 (serine), 66 (glycine), 67 (serine), 68 (glycine), 69 (threonine), 70 (aspartate), 71 (phenylalanine), 73 (leucine), 74 (threonine), 75 (threonine), 76 (serine), 77 (serine), 79 (glutamine), 81 (glutamate), 82 (aspartate), 85 (aspartate), 86 (tyrosine), 87 (tyrosine), 88 (cysteine), 90 (glutamine), 95 (proline), 97 (threonine), 98 (phenylalanine), 99 (glycine), 101 (glycine), 102 (threonine), 103 (lysine), 105 (glutamate/aspartate), or 107 (lysine) (linear numbering as in Figure 3B).

[0060] In one embodiment, the light chain immunoglobulin of the anti-PSMA antibody, or antigen-binding fragment thereof, includes one or more of:

a framework region 1 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, fourteen, fifteen amino acids selected from the group consisting of residue 2 (isoleucine), 4 (methionine), 5 (threonine), 6 (glutamine), 8 (proline), 10 (serine), 12 (serine), 14 (serine), 16 (glycine), 17 (glutamate/aspartate), 18 (arginine), 20 (threonine), 21 (leucine), 22 (threonine), and 23 (cysteine) (linear numbering as shown in Figure 3B);

a CDR1 having at least one, two, three, four, five, six, seven amino acids selected from the group consisting of residue 24 (lysine), 25 (alanine), 26 (serine), 29 (valine), 30 (glycine), 31 (threonine), and 33 (valine) (linear numbering as shown in Figure 3B);

a framework region 2 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve amino acids selected from the group consisting of residue 35 (tryptophan), 36 (tryrosine), 37 (glutamine), 38 (glutamine), 39 (lysine), 40 (proline), 43 (serine), 44 (proline), 45 (lysine), 47 (leucine), 48 (isoleucine), and 49 (tyrosine) (linear numbering as shown in Figure 3B);

a CDR2 having at least one, two, three, four amino acids selected from the group consisting of residue 51 (alanine), 52 (serine), 54 (arginine), and 56 (threonine) (linear numbering as shown in Figure 3B);

a framework region 3 having at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four amino acids selected from the group consisting of residue 59 (proline), 61 (arginine), 62 (phenylalanine), 63 (serine), 64 (glycine), 65 (serine), 66 (glycine), 67 (serine), 68 (glycine), 69 (threonine), 70 (aspartate), 71 (phenylalanine), 73 (leucine), 74 (threonine), 75 (threonine), 76 (serine), 77 (serine), 79 (glutamine), 81 (glutamate), 82 (aspartate), 85 (aspartate), 86 (tyrosine), 87 (tyrosine), and 88 (cysteine) (linear numbering as shown in Figure 3B);

a CDR3 having at least one, two, three, four amino acids selected from the group consisting of residue 90 (glutamine), 95 (proline), 97 (threonine), and 98 (phenylalanine) (linear numbering as shown in Figure 3B); or

a framework region 4 having at least one, two, three, four, five, six amino acid selected from the group consisting of residue 99 (glycine), 101 (glycine), 102 (threonine), 103 (lysine), 105 (glutamate/aspartate), and 107 (lysine) (linear numbering as shown in Figure 3B).

[0061] An anti-PSMA antibody, e.g., a modified anti-PSMA antibody, or antigen-binding fragment thereof, described herein can be used alone, e.g., can be administered to a subject, or used *in vitro*, in non-derivatized or unconjugated forms. In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, can be derivatized or linked to another molecular entity, typically a label or a therapeutic (e.g., a cytotoxic or cytostatic) agent. The molecular entity can be, e.g., another peptide, protein (including, e.g., a viral coat protein of, e.g., a recombinant viral particle), a non-peptide chemical compound, isotope, etc. The anti-PSMA antibody, or antigen-binding fragment thereof, can be functionally linked, e.g., by chemical coupling, genetic fusion, non-covalent association or otherwise, to one or more other molecular entities. For example, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a label, such as a fluorescent label, a biologically active enzyme label, a radioisotope (e.g., a radioactive ion), a nuclear magnetic resonance active label, a luminescent label, or a chromophore. In other embodiments, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a therapeutic agent, e.g., a cytotoxic moiety, e.g., a therapeutic drug, a radioisotope, molecules of plant, fungal, or bacterial origin, or biological proteins (e.g., protein toxins) or particles (e.g., recombinant viral particles, e.g., via a viral coat protein), or mixtures thereof. The therapeutic agent can be an intracellularly active drug or other agent, such as short-range radiation emitters, including, for example, short-range, high-energy α -emitters, as described herein. In some preferred embodiments, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or the DM1 maytansinoid, see Fig. 15), a taxane, or a calicheamicin. A radioisotope can be an α -, β -, or γ -emitter, or an β - and γ -emitter. Radioisotopes useful as therapeutic agents include yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), and rhodium (^{188}Rh). Radioisotopes useful as labels, e.g., for use in diagnostics, include iodine (^{131}I or ^{125}I), indium (^{111}In), technetium ($^{99\text{m}}\text{Tc}$), phosphorus (^{32}P), carbon (^{14}C), and tritium (^3H), or one of the therapeutic isotopes listed above. The anti-PSMA antibody, or antigen-binding fragment

thereof can also be linked to another antibody to form, e.g., a bispecific or a multispecific antibody.

[0062] In another aspect, the invention features, an anti-PSMA antibody, e.g., an antibody described herein, coupled, e.g., by covalent linkage, to a proteasome inhibitor or a topoisomerase inhibitor. [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-[(3-mercaptoacetyl)amino]propyl]amino]butyl] Boronic acid is a suitable proteasome inhibitor. N,N'-bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine is a suitable topoisomerase inhibitor.

[0063] In another aspect, the invention provides, compositions, e.g., pharmaceutical compositions, which include a pharmaceutically acceptable carrier, excipient or stabilizer, and at least one of the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies (or fragments thereof) described herein. In a preferred embodiment the antibody is conjugated to a label or a therapeutic agent. In one embodiment, the compositions, e.g., the pharmaceutical compositions, comprise a combination of two or more of the aforesaid anti-PSMA antibodies. For example, a composition, e.g., pharmaceutical composition, which comprises a deimmunized J591 antibody, in combination with another anti-PSMA antibody, or an antibody to another tumor cell-associated antigen, e.g., EGF receptor, Her-2/neu, etc. Combinations of the anti-PSMA antibody and a drug, e.g., a therapeutic agent (e.g., a cytotoxic or cytostatic drug, e.g., DM1, calicheamicin, or taxanes, topoisomerase inhibitors, or an immunomodulatory agent, e.g., IL-1, 2, 4, 6, or 12, interferon alpha or gamma, or immune cell growth factors such as GM-CSF) are also within the scope of the invention.

[0064] The invention also features nucleic acid sequences that encode a heavy and light chain immunoglobulin described herein. For example, the invention features, a first and second nucleic acid encoding a modified heavy and light chain variable region, respectively, of a modified anti-PSMA antibody molecule as described herein. In another aspect, the invention features host cells and vectors containing the nucleic acids of the invention.

[0065] In another aspect, the invention features a method of producing an anti-PSMA antibody, e.g., a modified anti-PSMA antibody, or antigen-binding fragment thereof. The method includes:

providing a first nucleic acid encoding a heavy chain variable region, e.g., a modified heavy chain variable region as described herein;

providing a second nucleic acid encoding a light chain variable region, e.g., a modified light chain variable region as described herein; and

introducing said first and second nucleic acids into a host cell under conditions that allow expression and assembly of said light and heavy chain variable regions.

[0066] The first and second nucleic acids can be linked or unlinked, e.g., expressed on the same or different vector, respectively.

[0067] The host cell can be a eukaryotic cell, e.g., a mammalian cell, an insect cell, a yeast cell, or a prokaryotic cell, e.g., *E. coli*. For example, the mammalian cell can be a cultured cell or a cell line. Exemplary mammalian cells include lymphocytic cell lines (e.g., NS0), Chinese hamster ovary cells (CHO), COS cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cell. For example, nucleic acids encoding the modified antibody described herein can be expressed in a transgenic animal. In one embodiment, the nucleic acids are placed under the control of a tissue-specific promoter (e.g., a mammary specific promoter) and the antibody is produced in the transgenic animal. For example, the antibody molecule is secreted into the milk of the transgenic animal, such as a transgenic cow, pig, horse, sheep, goat or rodent.

[0068] The invention also features a method of ablating or killing, a cell, e.g., a prostatic cell (e.g., a cancerous or non-cancerous prostatic cell, e.g., a normal, benign or hyperplastic prostatic epithelial cell), or a malignant, non-prostatic cell, e.g., cell found in a non-prostatic solid tumor that, e.g., has vasculature which expresses PSMA, a soft tissue tumor, or a metastatic lesion (e.g., a cell found in renal, urothelial (e.g. bladder), colonic, rectal, pulmonary, breast or hepatic cancers and/or metastases thereof). Methods of the invention include contacting the cell, or a nearby cell, e.g., a vascular endothelial cell proximate to the cell, with an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, in an amount sufficient to ablate or kill, the cell. Alternatively, an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, preferably a fragment of a modified anti-PSMA antibody, can be conjugated to a viral particle, e.g., to a coat protein of a viral particle. The anti-PSMA/viral particle conjugate can be used to target prostate cells, e.g., cancerous prostate cells, with genetically engineered viral particles that infect the cells and express, e.g., pro apoptotic genes, to thereby kill the cells or inhibit cell growth.

[0069] The methods can be used on cells in culture, e.g. *in vitro* or *ex vivo*. For example, prostatic cells (e.g., malignant or normal, benign or hyperplastic prostate epithelial cells) or non-prostatic cancerous or metastatic cells (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells) can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the modified anti-PSMA antibody or fragment thereof, to the culture medium. The method can be performed on cells (e.g., prostatic cells, or non-prostatic cancerous or metastatic cells) present in a subject, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol.

[0070] Methods of the invention can be used, for example, to treat or prevent a disorder, e.g., a prostatic disorder (e.g., a cancerous or non-cancerous prostatic disorder, e.g., a benign or hyperplastic prostatic disorder), or a non-prostatic disorder (e.g., cancer, e.g., malignant cancer), by administering to a subject an antibody described herein, preferably a modified PSMA antibody, or antigen-binding fragment thereof, in an amount effective to treat or prevent such disorder. Particularly preferred antibodies include modified antibodies having CDRs from any of a J591, J415, J533 or E99, and in particular deimmunized versions of these antibodies, particularly deJ591 or deJ415. Examples of prostatic disorders that can be treated or prevented include, but are not limited to, genitourinary inflammation (e.g., inflammation of smooth muscle cells) as in prostatitis; benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or hyperplasia); and cancer, e.g., adenocarcinoma or carcinoma, of the prostate and/or testicular tumors. Methods and compositions disclosed herein are particularly useful for treating metastatic lesions associated with prostate cancer. In some embodiments, the patient will have undergone one or more of prostatectomy, chemotherapy, or other anti-tumor therapy and the primary or sole target will be metastatic lesions, e.g., metastases in the bone marrow or lymph nodes. Examples of non-prostatic cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and particularly metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), genitals and genitourinary tract (e.g., renal, urothelial, bladder cells), pharynx, CNS (e.g., neural or glial cells), skin (e.g., melanoma), and pancreas, as well as adenocarcinomas which include

malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Methods and compositions disclosed herein are particularly useful for treating metastatic lesions associated with the aforementioned cancers. In some embodiments, the patient will have undergone one or more of surgical removal of a tissue, chemotherapy, or other anti-cancer therapy and the primary or sole target will be metastatic lesions, e.g., metastases in the bone marrow or lymph nodes.

[0071] In a preferred embodiment the subject is treated to prevent a disorder, e.g., a prostatic disorder. The subject can be one at risk for the disorder, e.g., a subject having a relative afflicted with the disorder, e.g., a subject with one or more of a grandparent, parent, uncle or aunt, sibling, or child who has or had the disorder, or a subject having a genetic trait associated with risk for the disorder. In a preferred embodiment the disorder is a prostatic disorder (e.g., a cancerous or non-cancerous prostatic disorder, e.g., a benign or hyperplastic prostatic disorder), or a non-prostatic disorder (e.g., cancer, e.g., malignant cancer) and the subject has one or more of a grandfather, father, uncle, brother, or son who has or had the disorder, or a subject having a genetic trait associated with risk for the disorder.

[0072] The subject can be a mammal, e.g., a primate, preferably a higher primate, e.g., a human (e.g., a patient having, or at risk of, a disorder described herein, e.g., a prostatic or a cancer disorder). In one embodiment, the subject is a patient having prostate cancer (e.g., a patient suffering from recurrent or metastatic prostate cancer).

[0073] The modified anti-PSMA antibody or fragment thereof, e.g., a modified anti-PSMA antibody or fragment thereof as described herein, can be administered to the subject systemically (e.g., orally, parenterally, subcutaneously, intravenously, rectally, intramuscularly, intraperitoneally, intranasally, transdermally, or by inhalation or intracavitary installation), topically, or by application to mucous membranes, such as the nose, throat and bronchial tubes.

[0074] The methods of the invention, e.g., methods of treatment or preventing, can further include the step of monitoring the subject, e.g., for a change (e.g., an increase or decrease) in one or more of: tumor size; levels of a cancer marker, e.g., levels of PSA, alkaline phosphatase, or serum hemoglobin for a patient with prostate cancer; the rate of appearance of new lesions, e.g., in a bone scan; the appearance of new disease-related symptoms; the size of soft tissue mass, e.g., a decreased or stabilization; quality of life, e.g., amount of disease

associated pain, e.g., bone pain; or any other parameter related to clinical outcome. The subject can be monitored in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered.

Monitoring can be used to evaluate the need for further treatment with the same modified anti-PSMA antibody or fragment thereof or for additional treatment with additional agents.

Generally, a decrease in one or more of the parameters described above is indicative of the improved condition of the subject, although with serum hemoglobin levels, an increase can be associated with the improved condition of the subject.

[0075] The methods of the invention can further include the step of analyzing a nucleic acid or protein from the subject, e.g., analyzing the genotype of the subject. In one embodiment, a nucleic acid encoding human PSMA and/or an upstream or downstream component(s) of human PSMA signalling, e.g., an extracellular or intracellular activator or inhibitor of human PSMA, is analyzed. The analysis can be used, e.g., to evaluate the suitability of, or to choose between alternative treatments, e.g., a particular dosage, mode of delivery, time of delivery, inclusion of adjunctive therapy, e.g., administration in combination with a second agent, or generally to determine the subject's probable drug response phenotype or genotype. The nucleic acid or protein can be analyzed at any stage of treatment, but preferably, prior to administration of the modified anti-PSMA antibody or fragment thereof to thereby determine appropriate dosage(s) and treatment regimen(s) of the modified anti-PSMA antibody or fragment thereof (e.g., amount per treatment or frequency of treatments) for prophylactic or therapeutic treatment of the subject.

[0076] The modified anti-PSMA antibody or fragment thereof can be used alone in unconjugated form to thereby ablate or kill the PSMA-expressing prostatic or cancerous cells by, e.g., antibody-dependent cell killing mechanisms such as complement-mediated cell lysis and/or effector cell-mediated cell killing. In other embodiments, the modified anti-PSMA antibody or fragment thereof can be bound to a substance, e.g., a cytotoxic agent or moiety, e.g., a therapeutic drug, a compound emitting radiation, molecules of plant, fungal, or bacterial origin, or a biological protein (e.g., a protein toxin) or particle (e.g., a recombinant viral particle, e.g., via a viral coat protein). For example, the modified anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a radioactive isotope such as an α -, β -, or γ -emitter, or a β - and γ -emitter. Examples of radioactive isotopes include iodine (^{131}I or ^{125}I), yttrium (^{90}Y),

lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, or bismuth (^{212}Bi or ^{213}Bi). Alternatively, the anti-PSMA antibody, or antigen-binding fragment thereof, can be coupled to a biological protein, a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or DM1), as well as a taxane (e.g., taxol or taxotere), or calicheamicin. The maytansinoid can be, for example, maytansinol or a maytansinol analogue. Examples of maytansinol analogues include those having a modified aromatic ring (e.g., C-19-dechloro, C-20-demethoxy, C-20-acyloxy) and those having modifications at other positions (e.g., C-9-CH₃, C-14-alkoxymethyl, C-14-hydroxymethyl or aceloxymethyl, C-15-hydroxy/acyloxy, C-15-methoxy, C-18-N-demethyl, 4,5-deoxy). Maytansinol and maytansinol analogues are described, for example, in U.S. Patent Number 6,333,410, the contents of which is incorporated herein by reference. The calicheamicin can be, for example, a bromo-complex calicheamicin (e.g., an alpha, beta or gamma bromo-complex), an iodo-complex calicheamicin (e.g., an alpha, beta or gamma iodo-complex), or analogs and mimics thereof. Bromo-complex calicheamicins include $\alpha_1\text{-BR}$, $\alpha_2\text{-BR}$, $\alpha_3\text{-BR}$, $\alpha_4\text{-BR}$, $\beta_1\text{-BR}$, $\beta_2\text{-BR}$ and $\gamma_1\text{-BR}$. Iodo-complex calicheamicins include $\alpha_1\text{-I}$, $\alpha_2\text{-I}$, $\alpha_3\text{-I}$, $\beta_1\text{-I}$, $\beta_2\text{-I}$, $\delta_1\text{-I}$ and $\gamma_1\text{-BR}$. Calicheamicin and mutants, analogs and mimics thereof are described, for example, in U.S. Patent Numbers 4,970,198, issued November 13, 1990, 5,264,586, issued November 23, 1993, 5,550,246, issued August 27, 1996, 5,712,374, issued January 27, 1998, and 5,714,586, issued February 3, 1998, the contents of which are incorporated herein by reference. Maytansinol can be coupled to antibodies using, e.g., an N-succinimidyl 3-(2-pyridyldithio)propionate (also known as N-succinimidyl 4-(2-pyridyldithio)pentanoate or SPP), 4-succinimidyl-oxycarbonyl-a-(2-pyridyldithio)-toluene (SMPT), N-succinimidyl-3-(2-pyridyldithio)butyrate (SDPB), 2-iminothiolane, or S-acetylsuccinic anhydride.

[0077] The methods and compositions of the invention can be used in combination with other therapeutic modalities. In one embodiment, the methods of the invention include administering to the subject a modified anti-PSMA antibody or fragment thereof, e.g., a modified anti-PSMA antibody or fragment thereof as described herein, in combination with a cytotoxic agent, in an amount effective to treat or prevent said disorder. The antibody and the cytotoxic agent can be administered simultaneously or sequentially. In other embodiments, the methods and compositions of the invention are used in combination with surgical and/or radiation procedures. In yet other embodiments, the methods can be used in combination with

immunomodulatory agents, e.g., IL-1, 2, 4, 6, or 12, or interferon alpha or gamma, or immune cell growth factors such as GM-CSF.

[0078] Exemplary cytotoxic agents that can be administered in combination with the anti-PSMA antibodies include antimicrotubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis and radiation.

[0079] In therapies of prostatic disorders, e.g., prostate cancer, the anti-PSMA antibodies can be used in combination with existing therapeutic modalities, e.g., prostatectomy (partial or radical), radiation therapy, hormonal therapy, androgen ablation therapy, and cytotoxic chemotherapy. Typically, hormonal therapy works to reduce the levels of androgens in a patient and can involve administering a leuteinizing hormone-releasing hormone (LHRH) analog or agonist (e.g., Lupron, Zoladex, leuprolide, buserelin, or goserelin), as well as antagonists (e.g., Abarelix). Non-steroidal anti-androgens, e.g., flutamide, bicalutimide, or nilutamide, can also be used in hormonal therapy, as well as steroidal anti-androgens (e.g., cyproterone acetate or megestrol acetate), estrogens (e.g., diethylstilbestrol), surgical castration, PROSCAR™, secondary or tertiary hormonal manipulations (e.g., involving corticosteroids (e.g., hydrocortisone, prednisone, or dexamethasone), ketoconazole, and/or aminoglutethimide), inhibitors of 5 α -reductase (e.g., finasteride), herbal preparations (e.g., PC-SPES), hypophysectomy, and adrenalectomy. Furthermore, hormonal therapy can be performed intermittently or using combinations of any of the above treatments, e.g., combined use of leuprolide and flutamide.

[0080] Any combination and sequence of modified anti-PSMA and other therapeutic modalities can be used. The modified anti-PSMA and other therapeutic modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The modified anti-PSMA and other therapeutic modalities can be administered before treatment, concurrently with treatment, posttreatment, or during remission of the disorder.

[0081] In another aspect, the invention features methods for detecting the presence of a PSMA protein in a sample *in vitro* (e.g., a biological sample, e.g., serum, semen or urine, or a tissue biopsy, e.g., from a prostatic or cancerous lesion). The subject method can be used to evaluate, e.g., diagnose or stage a disorder described herein, e.g., a prostatic or cancerous disorder. The method includes: (i) contacting the sample (and optionally, a reference, e.g., a

control sample) with a modified anti-PSMA antibody, or fragment thereof, as described herein, under conditions that allow interaction of the anti-PSMA antibody and the PSMA protein to occur; and (ii) detecting formation of a complex between the anti-PSMA antibody, and the sample (and optionally, the reference, e.g., control, sample). Formation of the complex is indicative of the presence of PSMA protein, and can indicate the suitability or need for a treatment described herein. For example, a statistically significant change in the formation of the complex in the sample relative to the reference sample, e.g., the control sample, is indicative of the presence of PSMA in the sample. In some embodiments, the methods can include the use of more than one anti-PSMA antibody, e.g., two anti-PSMA antibodies that bind to different epitopes on PSMA. For example, the method can involve an ELISA assay, e.g., as described in Example 19.

[0082] In yet another aspect, the invention provides a method for detecting the presence of PSMA *in vivo* (e.g., *in vivo* imaging in a subject). The method can be used to evaluate, e.g., diagnose or stage a disorder described herein, e.g., a prostatic or a cancerous disorder, in a subject, e.g., a mammal, e.g., a primate, e.g., a human. The method includes: (i) administering to a subject a modified anti-PSMA antibody (or antigen binding fragment thereof), under conditions that allow interaction of the modified anti-PSMA antibody (or fragment thereof) and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA. A statistically significant change in the formation of the complex in the subject relative to the reference, e.g., the control subject or subject's baseline, is indicative of the presence of the PSMA.

[0083] In other embodiments, a method of diagnosing or staging a disorder as described herein (e.g., a prostatic or cancerous disorder) is provided. The method includes: (i) identifying a subject having, or at risk of having, the disorder; (ii) obtaining a sample of a tissue or cell affected with the disorder; (iii) contacting said sample or a control sample with an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody or fragment, under conditions that allow interaction of the binding agent and the PSMA protein to occur, and (iv) detecting formation of a complex. A statistically significant increase in the formation of the complex between the antibody (or fragment thereof) with respect to a reference sample, e.g., a control sample, is indicative of the disorder or the stage of the disorder.

[0084] Preferably, the modified anti-PSMA antibody or fragment thereof, used in the *in vivo* and *in vitro* diagnostic methods is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding agent. Suitable detectable substances include various biologically active enzymes, prosthetic groups, fluorescent materials, luminescent materials, paramagnetic (e.g., nuclear magnetic resonance active) materials, and radioactive materials. In some embodiments, the modified anti-PSMA antibody or fragment thereof is coupled to a radioactive ion, e.g., indium (^{111}In), iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), bismuth (^{212}Bi or ^{213}Bi), sulfur (^{35}S), carbon (^{14}C), tritium (^3H), rhodium (^{188}Rh), technetium ($^{99\text{m}}\text{Tc}$), praseodymium, or phosphorous (^{32}P).

[0085] In another aspect, the invention provides a method for determining the dose, e.g., radiation dose, that different tissues are exposed to when a subject, e.g., a human subject, is administered an anti-PSMA antibody that is conjugated to a radioactive isotope. The method includes: (i) administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, that is labeled with a radioactive isotope, e.g., ^{111}In , to a subject; (ii) measuring the amount of radioactive isotope located in different tissues, e.g., prostate, liver, kidney, or blood, at various time points until most, e.g., 50%, 80%, 90%, 95%, or more, of the radioactive isotope has been eliminated from the body of the subject; and (iii) calculating the total dose of radiation received by each tissue analyzed. In some embodiments, the measurements are taken at scheduled time points, e.g., day 1, 2, 3, 5, 7, and 12, following administration (at day 0) of the radioactively labeled anti-PSMA antibody to the subject. In some embodiments, the radiation dose that a tissue receives for one radioactive isotope, e.g., a gamma-emitter, e.g., ^{111}In , can be used to calculate the expected dose that the same tissue would receive from a different radioactive isotope, e.g., a beta-emitter, e.g., ^{90}Y .

[0086] In another aspect, the invention features methods of treating pain, e.g., reducing pain, experienced by a subject having or diagnosed with prostate disease, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The methods include administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, to a subject in an amount sufficient to treat, e.g., reduce, the

pain associated with prostate disease or non-prostate cancer. In some embodiments, the subject may have no signs of prostate disease or non-prostate cancer other than, e.g., elevated levels of serum PSA and the sensation of pain. The pain can be bone pain, as well as, pain associated with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. In addition, by reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[0087] In some embodiments, the modified anti-PSMA antibody is administered in an unconjugated form in an amount sufficient to treat, e.g., reduce, pain associated with prostate disease or non-prostate cancer. In other embodiments, the modified anti-PSMA antibody, or antigen-binding fragment thereof, is administered in a derivatized form, e.g., linked to another functional molecule, as described herein.

[0088] Other features and advantages of the instant invention will become more apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] *Figures 1A-1B* depict the amino acid sequence of murine J591 heavy and light chain variable region, respectively. The location of the CDRs is indicated in the Figures; the amino acid numbering is according to the Kabat numbering (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Note that the CDRs are considered to encompass the Chothia loops and the Kabat hypervariable regions together and the sequences have been annotated accordingly. Heavy Chain: CDR1 is depicted in SEQ ID NO:1; CDR2 is depicted in SEQ ID NO:2; CDR3 is depicted in SEQ ID NO:3; the framework excluding CDR regions is depicted in SEQ ID NO:7; and the framework including CDR regions is depicted in SEQ ID NO:19. Light Chain: CDR1 is depicted in SEQ ID NO:4; CDR2 is depicted in SEQ ID NO:5; CDR3 is depicted in SEQ ID NO:6; the framework excluding CDR regions is depicted in SEQ ID NO:8; and the framework including CDR regions is depicted in SEQ ID NO:20.

[0090] *Figures 2A-2B* depict the amino acid sequence of the deimmunized J591 heavy and light chain variable region, respectively. The location of the CDRs is indicated in the Figures; the amino acid numbering is according the Kabat numbering (see, Kabat, E.A., *et al.* (1991) *supra*). Note that the CDRs are considered to encompass the Chothia loops and the Kabat hypervariable regions together and the sequences have been annotated accordingly. Heavy Chain: CDR1 is depicted in SEQ ID NO:1; CDR2 is depicted in SEQ ID NO:2; CDR3 is depicted in SEQ ID NO:3; framework 1 is depicted in SEQ ID NO:9; framework 2 is depicted in SEQ ID NO:10; framework 3 is depicted in SEQ ID NO:11; framework 4 is depicted in SEQ ID NO:12; the framework excluding CDR regions is depicted in SEQ ID NO:17; and the framework including CDR regions is depicted in SEQ ID NO:21. Light Chain: CDR1 is depicted in SEQ ID NO:4; CDR2 is depicted in SEQ ID NO:5; CDR3 is depicted in SEQ ID NO:6; framework 1 is depicted in SEQ ID NO:13; framework 2 is depicted in SEQ ID NO:14; framework 3 is depicted in SEQ ID NO:15; framework 4 is depicted in SEQ ID NO:16; the framework excluding CDR regions is depicted in SEQ ID NO:18; and the framework including CDR regions is depicted in SEQ ID NO:22.

[0091] *Figures 3A-3B* depict an alignment of the murine J591 and deimmunized heavy chain variable regions (3A; SEQ ID NO:19 and 21, respectively) and light chain variable regions (3B; SEQ ID NO:20 and 22, respectively). Potential T cell epitopes (identified using a peptide threading program) in murine J591 VH and VK are shown in Figures 3A-3B, respectively. The 13-mer peptides predicted to bind to MHC class II are indicated by the underline; the CDRs are located at residues 26 to 35, 50 to 66, and 99-104 of Figure 3A and residues 24 to 34, 50 to 56, and 89 to 97 of Figure 3B; and residues altered in the deimmunized heavy and light chain variable regions are boxed. Where possible, amino acid substitutions are those commonly used in human germline VH regions. The amino acid numbering is linear, not according to Kabat.

[0092] *Figures 4A-4B* depict the nucleotide sequences of the deimmunized J591 heavy and light chain variable region, respectively. Figure 4A shows an alignment of the coding and noncoding nucleotide strands of deimmunized J591 heavy chain variable region (SEQ ID NOs:23 and 24, respectively) with the corresponding amino acid sequence (SEQ ID NO:27). Figure 4B shows an alignment of the coding and noncoding nucleotide strands of deimmunized J591 light chain variable region (SEQ ID NOs:25 and 26, respectively) with the corresponding

amino acid sequence (SEQ ID NO:28). The location of the signal peptide and CDRs 1-3 is indicated in each alignment.

[0093] *Figure 5* depicts an alignment of the amino acid sequences for the murine and several deimmunized heavy chain variable regions of the J415 antibody. The murine amino acid sequence is shown as J415VH (SEQ ID NO:47); the deimmunized sequences are depicted as J415DIVH1 (amino acid residues 18 to 133 of SEQ ID NO:54), J415DIVH2 (SEQ ID NO:59), J415DIVH3 (SEQ ID NO:60), and J415DIVH4 (SEQ ID NO:49). The preferred sequence is J415DIVH4 (SEQ ID NO:49). The amino acid replacements are indicated by the boxed residues. A consensus sequence is labeled "majority" (SEQ ID NO:61).

[0094] *Figure 6* depicts an alignment of the amino acid sequences for the murine and several deimmunized light chain variable regions of the J415 antibody. The murine amino acid sequence is shown as J415VK (SEQ ID NO:48); the deimmunized sequences are depicted as J415DIVK1 (amino acid residues 18 to 124 of SEQ ID NO:57), J415DIVK2 (SEQ ID NO:62), J415DIVK3 (SEQ ID NO:63), J415DIVK4 (SEQ ID NO:64), J415DIVK5 (SEQ ID NO:50), J415DIVK6 (SEQ ID NO:65), J415DIVK7 (SEQ ID NO:66), and J415DIVK8 (SEQ ID NO:67). The preferred sequence is J415DIVK5 (SEQ ID NO:50). The amino acid replacements are indicated by the boxed residues. A consensus sequence is labeled "majority" (SEQ ID NO:68).

[0095] *Figure 7A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the deimmunized J415 heavy chain variable region (J415DIVH1) (SEQ ID NO:53-55, respectively). The relative location of the signal sequence, intron and J415DIVH1 amino acid sequence is indicated, as well as some restriction sites.

[0096] *Figure 7B* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J415 heavy chain variable region (SEQ ID NO:125, 47, and 126, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[0097] *Figure 7C* depicts an alignment of the amino acid sequence of the murine J415 heavy chain variable region (SEQ ID NO:47) and a consensus sequence for Kabat subgroup murine VHIII (MUVHIII, SEQ ID NO:69). A consensus majority sequence based on the alignment is also shown (SEQ ID NO:70).

[0098] *Figure 8A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the deimmunized J415 light chain variable region (J415DIVK1) (SEQ ID NO:56-58, respectively). The relative location of the signal sequence, intron and J415DIVK1 amino acid sequence is indicated, as well as some restriction sites.

[0099] *Figure 8B* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J415 light chain variable region (SEQ ID NOs:127, 48, and 128, respectively). The relative locations of the CDRs and some restriction sites are also indicated.

[00100] *Figure 8C* depicts an alignment of the amino acid sequence of the murine J415 light chain variable region (SEQ ID NO:48) and a consensus sequence for Kabat subgroup murine variable light chain (MuVKI, SEQ ID NO:71). A consensus majority sequence based on the alignment is also shown (SEQ ID NO:72).

[00101] *Figure 9A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J533 heavy chain variable region (SEQ ID NO:73-75, respectively). The relative locations of the CDRs and restriction sites are indicated.

[00102] *Figure 9B* depicts an alignment of the amino acid sequence of the murine J533 heavy chain variable region (SEQ ID NO:74) and a consensus sequence for Kabat subgroup murine variable heavy chain (MuVHIIA, SEQ ID NO:79). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:80).

[00103] *Figure 10A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine J533 light chain variable region (SEQ ID NO:76-78, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00104] *Figure 10B* depicts an alignment of the amino acid sequence of the murine J533 light chain variable region (SEQ ID NO:77) and a consensus sequence for Kabat subgroup murine MuVKIII, SEQ ID NO:81). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:82).

[00105] *Figure 11A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine E99 heavy chain variable

region (SEQ ID NO:83-85, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00106] *Figure 11B* depicts an alignment of the amino acid sequence of the murine E99 heavy chain variable region (SEQ ID NO:84) and a consensus sequence for Kabat subgroup murine variable heavy chain (MuVHIB, SEQ ID NO:89). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:90).

[00107] *Figure 12A* depicts the nucleic acid coding sequence, the amino acid sequence, and the nucleic acid reverse complement sequence of the murine E99 light chain variable region (SEQ ID NO:86-88, respectively). The relative locations of the CDRs and some restriction sites are indicated.

[00108] *Figure 12B* depicts an alignment of the amino acid sequence of the murine E99 light chain variable region (SEQ ID NO:87) and a consensus sequence for Kabat subgroup murine variable light chain (MuVKI, SEQ ID NO:91). A consensus majority sequence based upon the alignment is also shown (SEQ ID NO:92).

[00109] *Figures 13A and B* depict serum PSA levels as a function of time for two patients that were treated with a single dose of ^{90}Y -DOTA-deJ591. Day 0 corresponds to the day on which the ^{90}Y -DOTA-deJ591 was administered.

[00110] *Figure 14* depicts the serum PSA levels as a function of time for a patient that was treated with a single dose of ^{177}Lu -DOTA-deJ591. Day 0 corresponds to the day on which the ^{177}Lu -DOTA-deJ591 was administered.

[00111] *Figure 15* depicts the chemical structures of DM1 and maytansine, a related molecule that lacks the thiol reactive group of DM1 used to conjugate DM1 to antibodies.

DETAILED DESCRIPTION OF THE INVENTION

[00112] This invention provides, *inter alia*, antibodies, e.g., modified antibodies, or antigen-binding fragments thereof, to the extracellular domain of human prostate specific membrane antigen (PSMA). The modified anti-PSMA antibodies, or antigen-binding fragments thereof, have been rendered less immunogenic compared to their unmodified counterparts to a given species, e.g., a human. Human PSMA is expressed on the surface of normal, benign hyperplastic epithelial cells (e.g., benign prostate secretory-acinar epithelium), and cancerous prostate epithelial cells (e.g., prostatic intraepithelial neoplasia and prostatic adenocarcinoma), as

well as vascular endothelial cells proximate to cancerous cells, e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct), melanoma (e.g., malignant melanoma), or soft tissue sarcoma cancerous cells. The antibodies, e.g., the modified antibodies, of the invention bind to the cell surface of cells that express PSMA. PSMA is normally recycled from the cell membrane into the cell. Thus, the antibodies of the invention are internalized with PSMA through the process of PSMA recirculation, thereby permitting delivery of an agent conjugated to the antibody, e.g., a labeling agent, a cytotoxic agent, or a viral particle (e.g., a viral particle containing genes that encode cytotoxic agents, e.g., apoptosis-promoting factors).

[00113] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[00114] As used herein, "PSMA" or "prostate-specific membrane antigen" protein refers to mammalian PSMA, preferably human PSMA protein. Human PSMA includes the two protein products, PSMA and PSM', encoded by the two alternatively spliced mRNA variants (containing about 2,653 and 2,387 nucleotides, respectively) of the PSMA cDNA disclosed in Israeli *et al.* (1993) *Cancer Res.* 53:227-230; Su *et al.* (1995) *Cancer Res.* 55:1441-1443; US 5,538,866, US 5,935,818, and WO 97/35616, the contents of which are hereby incorporated by reference. The long transcript of PSMA encodes a protein product of about 100-120 kDa molecular weight characterized as a type II transmembrane receptor having sequence homology with the transferrin receptor and having NAALADase activity (Carter *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:749-753). Accordingly, the term "human PSMA" refers to at least two protein products, human PSMA and PSM', which have or are homologous to (e.g., at least about 85%, 90%, 95% identical to) an amino acid sequence as shown in Israeli *et al.* (1993) *Cancer Res.* 53:227-230; Su *et al.* (1995) *Cancer Res.* 55:1441-1443; US 5,538,866, US 5,935,818, and WO 97/35616; or which is encoded by (a) a naturally occurring human PSMA nucleic acid sequence (e.g., Israeli *et al.* (1993) *Cancer Res.* 53:227-230 or US 5,538,866); (b) a nucleic acid sequence degenerate to a naturally occurring human PSMA sequence; (c) a nucleic acid sequence homologous to (e.g., at least about 85%, 90%, 95% identical to) the naturally occurring human PSMA nucleic acid sequence; or (d) a nucleic acid sequence that hybridizes to one of the foregoing nucleic acid sequences under stringent conditions, e.g., highly stringent conditions.

[00115] An "anti-PSMA antibody" is an antibody that interacts with (e.g., binds to) PSMA, preferably human PSMA protein. Preferably, the anti-PSMA antibody interacts with, e.g., binds to, the extracellular domain of PSMA, e.g., the extracellular domain of human PSMA located at about amino acids 44-750 of human PSMA (amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866). In one embodiment, the anti-PSMA antibody binds all or part of the epitope of an antibody described herein, e.g., J591, E99, J415, and J533. The anti-PSMA antibody can inhibit, e.g., competitively inhibit, the binding of an antibody described herein, e.g., J591, E99, J415, and J533, to human PSMA. An anti-PSMA antibody may bind to an epitope, e.g., a conformational or a linear epitope, which epitope when bound prevents binding of an antibody described herein, J591, E99, J415, and J533. The epitope can be in close proximity spatially or functionally-associated, e.g., an overlapping or adjacent epitope in linear sequence or conformationally to the one recognized by the J591, E99, J415, or J533 antibody. In one embodiment, the anti-PSMA antibody binds to an epitope located wholly or partially within the region of about amino acids 120 to 500, preferably 130 to 450, more preferably, 134 to 437, or 153 to 347, of human PSMA (amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866). Preferably, the epitope includes at least one glycosylation site, e.g., at least one N-linked glycosylation site (e.g., an asparagine residue located at about amino acids 190-200, preferably at about amino acid 195, of human PSMA; amino acid residues correspond to the human PSMA sequence disclosed in US 5,538,866).

[00116] Cell lines that produce anti-PSMA antibodies, e.g., murine and modified anti-PSMA antibodies, described herein have been deposited with the ATCC. The ATCC designations of the cell lines that produce each of the anti-PSMA antibodies are listed in Table 7.

Table 7

Anti-PSMA Antibody	ATCC Designation
E99	HB-12101
J415	HB-12109
J533	HB-12127
J591	HB-12126
deJ591	PTA-3709
deJ415	PTA-4174

[00117] In a preferred embodiment, the interaction, e.g., binding, occurs with high affinity (e.g., affinity constant of at least 10^7 M^{-1} , preferably, between 10^8 M^{-1} and 10^{10} , or about 10^9 M^{-1}) and specificity. Preferably, the anti-PSMA antibody treats, e.g., ablates or kills, a cell, e.g., a PSMA-expressing cell (e.g., a prostatic or cancerous cell). The mechanism by which the anti-PSMA antibody treats, e.g., ablates or kills, the cell is not critical to the practice of the invention. In one embodiment, the anti-PSMA antibody may bind to and be internalized with the PSMA expressed in the cells and/or vascular endothelial cells proximate to the cells. In those embodiments, the anti-PSMA antibody can be used to target a second moiety, e.g., a labeling agent, a labeling agent, or a viral agent, to the cell. In other embodiments, the anti-PSMA antibody may mediate host-mediated-killing, e.g., complement- or ADCC-mediated killing, of the cell and/or the vascular cell proximate thereto, upon binding to the extracellular domain of PSMA. The cell can be killed directly by the anti-PSMA antibody by binding directly to the cell or the vascular endothelial cells proximate thereto. Alternatively, the anti-PSMA antibody can treat, e.g., kill or ablate, or otherwise change the properties of the vascular endothelial cells to which it binds so that blood flow to the cells proximate thereto is reduced, thereby causing the cells to be killed or ablated. Examples of anti-PSMA antibodies include, e.g., monospecific, monoclonal (e.g., human), recombinant or modified, e.g., chimeric, CDR-grafted, humanized, deimmunized, and *in vitro* generated anti-PSMA antibodies.

[00118] As used herein, the term "treat" or "treatment" is defined as the application or administration of an anti-PSMA antibody or antigen binding fragment thereof to a subject, e.g., a patient, or application or administration to an isolated tissue or cell from a subject, e.g., a patient, which is returned to the patient. The anti-PSMA antibody or antigen binding fragment thereof, can be administered alone or in combination with, a second agent. The subject can be a patient having a disorder (e.g., a disorder as described herein), a symptom of a disorder or a predisposition toward a disorder. The treatment can be to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. While not wishing to be bound by theory treating is believed to cause the inhibition, ablation, or killing of a cell *in vitro* or *in vivo*, or otherwise reducing capacity of a cell, e.g., an aberrant cell, to mediate a disorder, e.g., a disorder as described herein (e.g., a cancer or prostatic disorder).

[00119] As used herein, an amount of an anti-PSMA antibody effective to treat a disorder, or a "therapeutically effective amount" refers to an amount of the antibody which is effective, upon single or multiple dose administration to a subject, in treating a cell, e.g., a prostatic or cancer cell (e.g., a PSMA-expressing prostatic or cancer cell, or a vascular cell proximate thereto), or in prolonging curing, alleviating, relieving or improving a subject with a disorder as described herein beyond that expected in the absence of such treatment. As used herein, "inhibiting the growth" of the neoplasm refers to slowing, interrupting, arresting or stopping its growth and metastases and does not necessarily indicate a total elimination of the neoplastic growth.

[00120] As used herein, an amount of an anti-PSMA antibody effective to prevent a disorder, or a "a prophylactically effective amount" of the antibody refers to an amount of an anti-PSMA antibody, e.g., an anti-PSMA antibody as described herein, which is effective, upon single- or multiple-dose administration to the subject, in preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a cancer or prostatic disorder as described herein, or treating a symptom thereof.

[00121] The terms "induce", "inhibit", "potentiate", "elevate", "increase", "decrease" or the like, e.g., which denote quantitative differences between two states, refer to a difference, e.g., a statistically or clinically significant difference, between the two states. For example, "an amount effective to inhibit the proliferation of the PSMA-expressing hyperproliferative cells" means that the rate of growth of the cells will be different, e.g., statistically different, from the untreated cells.

[00122] As used herein, "specific binding" refers to the property of the antibody to: (1) to bind to PSMA, e.g., human PSMA protein, with an affinity of at least $1 \times 10^7 \text{ M}^{-1}$, and (2) preferentially bind to PSMA, e.g., human PSMA protein, with an affinity that is at least two-fold, 50-fold, 100-fold, 1000-fold, or more greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than PSMA.

[00123] As used herein, the term "antibody" refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed

"framework regions" (FR). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, which are incorporated herein by reference). Preferably, each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00124] The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda.

[00125] As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The term "immunoglobulin" includes an immunoglobulin having: CDRs from a non-human source, e.g., from a non-human antibody, e.g., from a mouse immunoglobulin or another non-human immunoglobulin, from a consensus

sequence, or from a sequence generated by phage display, or any other method of generating diversity; and having a framework that is less antigenic in a human than a non-human framework, e.g., in the case of CDRs from a non-human immunoglobulin, less antigenic than the non-human framework from which the non-human CDRs were taken. The framework of the immunoglobulin can be human, humanized non-human, e.g., a mouse, framework modified to decrease antigenicity in humans, or a synthetic framework, e.g., a consensus sequence. These are sometimes referred to herein as modified immunoglobulins. A modified antibody, or antigen binding fragment thereof, includes at least one, two, three or four modified immunoglobulin chains, e.g., at least one or two modified immunoglobulin light and/or at least one or two modified heavy chains. In one embodiment, the modified antibody is a tetramer of two modified heavy immunoglobulin chains and two modified light immunoglobulin chains.

[00126] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG) that is encoded by heavy chain constant region genes.

[00127] The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to a portion of an antibody which specifically binds to PSMA (e.g., human PSMA), e.g., a molecule in which one or more immunoglobulin chains is not full length but which specifically binds to PSMA (e.g., human PSMA protein). Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR) having sufficient framework to specifically bind, e.g., an antigen binding portion of a variable region. An antigen binding portion of a light chain variable region and an antigen binding portion of a heavy chain variable region, e.g., the two domains of the Fv fragment, VL and VH, can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be

encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[00128] The term "monospecific antibody" refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a "monoclonal antibody" or "monoclonal antibody composition," which as used herein refer to a preparation of antibodies or fragments thereof of single molecular composition.

[00129] The term "recombinant" antibody, as used herein, refers to antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, *in vitro* generated (e.g., by phage display) antibodies, and may optionally include constant regions derived from human germline immunoglobulin sequences.

[00130] As used herein, the term "substantially identical" (or "substantially homologous") is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have similar activities. In the case of antibodies, the second antibody has the same specificity and has at least 50% of the affinity of the same.

[00131] Calculations of "homology" between two sequences can be performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide

positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[00132] The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent homology between two amino acid sequences is determined using the Needleman and Wunsch (1970), *J. Mol. Biol.* 48:444-453, algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent homology between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[00133] As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more

washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

[00134] It is understood that the antibodies and antigen binding fragment thereof of the invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect desired biological properties, such as binding activity can be determined as described in Bowie, JU et al. (1990) *Science* 247:1306-1310. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[00135] A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change.

Anti-PSMA Antibodies

[00136] Many types of anti-PSMA antibodies, or antigen-binding fragments thereof, are useful in the methods of this invention. The antibodies can be of the various isotypes, including: IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA1, IgA2, IgD, or IgE. Preferably, the antibody is an IgG isotype, e.g., IgG1. The antibody molecules can be full-length (e.g., an IgG1 or IgG4 antibody) or can include only an antigen-binding fragment (e.g., a Fab, F(ab')₂, Fv or a single chain Fv fragment). These include monoclonal antibodies, recombinant antibodies, chimeric antibodies, humanized antibodies, deimmunized antibodies, and human antibodies, as well as antigen-binding fragments of the foregoing.

[00137] Monoclonal anti-PSMA antibodies can be used in the methods of the invention. Preferably, the monoclonal antibodies bind to the extracellular domain of PSMA (i.e., an epitope of PSMA located outside of a cell). Examples of preferred murine monoclonal antibodies to human PSMA include, but are not limited to, E99, J415, J533 and J591, which are produced by hybridoma cell lines having an ATCC Accession Number HB-12101, HB-12109, HB-12127, and HB-12126, respectively, all of which are disclosed in US 6,107,090 and US 6,136,311, the contents of which are expressly incorporated by reference. Most preferably, the murine monoclonal antibody is J591, produced by HB-12126.

[00138] Additional monoclonal antibodies to PSMA can be generated using techniques known in the art. Monoclonal antibodies can be produced by a variety of techniques, including conventional monoclonal antibody methodology e.g., the standard somatic cell hybridization technique of Kohler and Milstein, *Nature* 256: 495 (1975). See generally, Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

[00139] Useful immunogens for the purpose of this invention include PSMA (e.g., human PSMA)-bearing cells (e.g., a prostate tumor cell line, e.g., LNCap cells, or fresh or frozen prostate tumor cells); membrane fractions of PSMA-expressing cells (e.g., a prostate tumor cell line, e.g., LNCap cells, or fresh or frozen prostate tumor cells); isolated or purified PSMA, e.g., human PSMA protein (e.g., biochemically isolated PSMA, or a portion thereof, e.g., the extracellular domain of PSMA). Techniques for generating antibodies to PSMA are described in US 6,107,090, US 6,136,311, the contents of all of which are expressly incorporated by reference.

[00140] Human monoclonal antibodies (mAbs) directed against human proteins can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc.*

Natl. Acad. Sci. USA 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuailon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

[00141] Anti-PSMA antibodies or fragments thereof useful in the present invention may also be recombinant antibodies produced by host cells transformed with DNA encoding immunoglobulin light and heavy chains of a desired antibody. Recombinant antibodies may be produced by known genetic engineering techniques. For example, recombinant antibodies may be produced by cloning a nucleotide sequence, e.g., a cDNA or genomic DNA, encoding the immunoglobulin light and heavy chains of the desired antibody. The nucleotide sequence encoding those polypeptides is then inserted into expression vectors so that both genes are operatively linked to their own transcriptional and translational expression control sequences. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. Typically, both genes are inserted into the same expression vector. Prokaryotic or eukaryotic host cells may be used.

[00142] Expression in eukaryotic host cells is preferred because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to well known methods (Kim and Baldwin, "Specific Intermediates in the Folding Reactions of Small Proteins and the Mechanism of Protein Folding", *Ann. Rev. Biochem.* 51, pp. 459-89 (1982)). It is possible that the host cells will produce portions of intact antibodies, such as light chain dimers or heavy chain dimers, which also are antibody homologs according to the present invention.

[00143] It will be understood that variations on the above procedure are useful in the present invention. For example, it may be desired to transform a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for PSMA binding, e.g., the constant region may be modified by, for example, deleting specific amino acids. The molecules expressed from such truncated DNA molecules are useful in the methods of this invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are anti-PSMA antibody and the other heavy and light chain are specific for an antigen other than PSMA, or another epitope of PSMA.

[00144] Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

[00145] An antibody or an immunoglobulin chain can be humanized by methods known in the art. Once the murine antibodies are obtained, the variable regions can be sequenced. The location of the CDRs and framework residues can be determined (see, Kabat, E.A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917, which are incorporated herein by reference). The light and heavy chain variable regions can, optionally, be ligated to corresponding constant regions.

[00146] Murine anti-PSMA antibodies can be sequenced using art-recognized techniques. As an example, hybridomas expressing murine hybridomas J533, J415 and E99 were propagated in culture in RPMI 1640 medium supplemented with 10% fetal calf serum. The isotype of the antibodies secreted was confirmed as IgG1 κ , IgG1 κ , and IgG3 κ respectively. These monoclonal antibodies, like J591, bind to the external domain of prostate specific membrane antigen. J591, J533 and E99 recognize the same epitope, while J415 recognizes an independent epitope. Total RNA for each monoclonal was prepared from 10⁷ hybridoma cells. V_H and V_K cDNA was prepared using reverse transcriptase and mouse κ constant region and mouse IgG constant region primers. The first strand cDNAs were amplified by PCR using a variety of mouse signal sequence primers (6 for V_H and 7 for V_K). The amplified DNAs were gel-purified and cloned into the vector pT7Blue. The V_H and V_K clones obtained were screened for correct inserts by

PCR and the DNA sequence of selected clones determined by the dideoxy chain termination method (see Table 7).

[00147] The DNA and amino acid sequences for the heavy and light chain variable regions from J415 were obtained and are shown in Figures 7B (V_H) and 8B (V_K) (also, see Table 5). The location of the CDRs is shown. J415 V_H can be assigned to Mouse Heavy Chains Subgroup IIIC (Kabat EA *et al*; *ibid*). The sequence of J415 V_H compared to the consensus sequence for this subgroup is shown in Figure 7C. J415 V_K can be assigned to Mouse Kappa Chains Subgroup I (Kabat EA *et al*; *ibid*). The sequence of J415 V_K compared to the consensus sequence for this subgroup is shown in Figure 8C.

[00148] The DNA and amino acid sequences encoding the heavy and light chain variable regions J533 were obtained and are shown in Figures 9A (V_H) and 10A (V_K) (see also Table 5). The location of the CDRs is shown in each figure. J533 V_H can be assigned to Mouse Heavy Chains Subgroup IIA (Kabat EA *et al*; Sequences of proteins of Immunological Interest, US Department of Health and Human Services, 1991). The sequence of J533 V_H compared to the consensus sequence for this subgroup is shown in Figure 9B. J533 V_K can be assigned to Mouse Kappa Chains Subgroup III (Kabat EA *et al*; *ibid*). The sequence of J533 V_K compared to the consensus sequence for this subgroup is shown in Figure 10B.

[00149] The DNA and amino acid sequences of the heavy and light chain variable regions of E99 were obtained and are shown in Figures 11A (V_H) and 12A (V_K) (see also Table 5). The location of the CDRs is shown. E99 V_H can be assigned to Mouse Heavy Chains Subgroup IB (Kabat EA *et al*; *ibid*). The sequence of E99 V_H compared to the consensus sequence for this subgroup is shown in Figure 11B. E99 V_K can be assigned Mouse Kappa Chains Subgroup I (Kabat EA *et al*; *ibid*). The sequence of E99 V_K compared to the consensus sequence for this subgroup is shown in Figure 12B.

[00150] The amino acid sequence and nucleotide sequences encoding the variable region of antibodies J415, deJ415, J591, deJ591, J533 and E99 are provided below in Table 8.

Table 8: Antibody variable chain sequences

NAME	Organism	FIG.	SEQ ID NO:	SEQUENCE
V _H J415	Mus musculus	Fig. 7B	125	gaagtgaagcttgaggagtctggaggaggcttggtgcaacctgg aggatccatgaaactctcctgtgttgctctggattcacttcagtaat

				tactggatgaactgggtccgccagtctccagagaaggggcttgag tgggttgctgaaattagatcgcaatctaataatttgcacacattatg cggagtctgtgaaaggagggtcatctcaagagatgattcca agagtagtgtctacctgcaaatgaacaacttgagagctgaagaca ctggcatttattactgtaccaggcgatggaataatttctggggccaa ggcaccactctcacagtctctca
V _H Variable Region J591	Mus musculus	Fig. 1A	19	EVQLQQSGPELKKPGTSVRISCKTSGYFT EYTIHWVKQSHGKSLEWIGNINPNNGGTT YNQKFEDKATLTVDKSSSTAYMELRSLTS EDSAVYYCAAGWNFDYWGQGTTLTVSS
V _H J415 (complementar y strand of SEQ ID NO:125)	Mus musculus	Fig. 7B	126	tgaggagactgtgagagtgtgccttgccccagaaattattccat cgctgtgtacagtaataaatgccagtgtcttcagctctcaagtgttc atttcaggtagacactactcttgaatcatctcttgagatgagacc ctccctttcacagactccgcataatgtgtgcaaaattattagattgc gatciaatttcagcaaccactcaagccccttctctggagactggc ggaccagttcatccagtaattactgaaagtgaatccagaggcaa cacaggagagtttcatggatcctccaggttgaccaagcctctcc agactcctcaagcttcacttc
V _L J415	Mus musculus	Fig. 8B	127	aacattgtaatgacccaatttccaaatccatgtccattcagtagga gagagggtcaccttgacctgcaaggccagtgaagaatgtgggtact tatgtgtcctggtatcaacagaaaccagaacagttcctaagatgtt gatatacggggcatccaaccgggtcactgggtccccgatcgctt cacaggcagtgatctgcaacagattcattctgaccatcagcagt gtgcagactgaagacctgttagattattactgtggacagagttacac cttccgtacacgttcggagggggggaccaagctggaaatgaag
V _L Variable Region J591	Mus musculus	Fig. 1B	20	DIVMTQSHKFMSTSVGDRVSIICKASQDV GTAVDWYQQKPGQSPKLLIYWASTRHTG VPDRFTGSGSGTDFTLTITNVQSEDLADYF CQQYNSYPLTFGAGTMLDLK
V _L J415 (complementar y strand of SEQ ID NO:127)	Mus musculus	Fig. 8B	128	cttcatttcagcttggtccccctccgaacgtgtacggaaaggtgt aactctgtccacagtaataatctacaaggtcttcagctgtcacactg ctgatgtcagaatgaaatctgttcagatccactgcctgtgaagc gatcgggggacccagtgaaaccggttgatgccccgtatatcaaca tcttaggagactgttctgtttctgttgataccaggacacataagtac ccacattctcactggccttgcaaggtcaaggtgaccctctctctact gaaatggacatggatttgggaaattgggtcattacaatgtt
V _H Variable Region (Deimm) J591	Artificial - deimmunized heavy chain J591	Fig. 2A	21	EVQLVQSGPEVKKPGATVKISCKTSGYFT EYTIHWVKQAPGKGLEWIGNINPNNGGTT YNQKFEDKATLTVDKSTDTAYMELSSLR EDTAVYYCAAGWNFDYWGQGTTLTVSS
V _L Variable Region (Deimm) J591	Artificial - deimmunized light chain J591	Fig. 2B	22	DIQMTQSPSSLSTSVGDRVTLTCKASQDV GTAVDWYQQKPGSPKLLIYWASTRHTGI PSRFSGSGSGTDFTLTISLQPEDFADYYCQ QYNSYPLTFGPGTKVDIK
V _H Deimmunized J591 CDS (122- 166)	Artificial - deimmunized heavy chain J591	Fig. 4A	23	aagcttatgaatatgcaatcctctgaatctacatggtaaatataggt ttgtctataccacaaacagaaaacatgagatcacagtctctctac agttactgagcacacaggacctcaccatgggatggagctgtatcat cctctcttggttagcaacagctacaggttaaggggtcacagtagca ggcttgaggtctggacatatatatgggtgacaatgacatccatttg ccttctctccacaggtgtccactccgaggtccaactggatcagctct

& CDS (249-605)				ggacctgaagtgaagaagcctggggctacagtgaagatattcctg caagacttctggatacacattcactgaatataccatacactgggtga agcaggccccctggaaggccttgagtgattggaacatcaatc ctaacaatgggtgtaccacctaacaaggttcgaggacaagg ccacactaactgtagacaagtcaccgatacagcctacatggagc tcagcagcctaagatctgaggatactgcagctattattgtgcagct ggttggaaacttgactactggggccaaggaccctgtcacccgtct cctcaggtgagtccttacaacctctctcttctatcagcttaaatagat tttactgcatttgggggggaaatgtgtgtatctgaatttcagggtca tgaaggactaggacaccttgggagtcagaaagggtcattggga gccccggctgatgcagacagacatcctcagctccagactcatg gccagagatttataggatcc
V _H Deimmunized (complimentary strand of SEQ ID NO:23) J591	Artificial - deimmunized heavy chain J591	Fig. 4A	24	ggatcctataaatctctggccatgaagtctgggagctgaggatgtc tgtctgcatcagccccgggctcccaatgaccctttctgactccaag gtgtccctagtcttcagctgaaattcagatacacacatttcccc cccaacaaatgcagtaaaatctatttaagctgaatagaagagagag gttgaaggactcacctgaggagacgggtgagcagggctccctggc cccagtagtcaaagtccaaccagctgcacaataatagactgcagt atcctcagatcttaggtgctgagctccatgtaggctgtatcggtgg acttgtctacagttagtgtggcctgtcctcgaacttctgattgtaggt ggtagccaccattgttaggtgatgtttccaatccactcaaggccctt tccaggggctgttcacccagtgtaggttatattcagtgaaatgtgt atccagaagtcttcagagatatcttcactgtagcccaggcttctc acttcaggtccagactgtaccagttggacctggagtggaacct gtggagagaaaggcaaagtggatgtcattgtcacccatatatgt ccagacctcaagcctgtactgtgagccccctacctgtagctgttg taccagaagaggatgatacagctccatccatgggtgaggtcctgt gtgtcagtaactgtagagagaactgtgatctcatgttttctgttgt ggtagatagacaaacctatattaccatgtagattcagaggattgcata ttcataagctt
V _L Deimmunized J591 CDS (122-166) & CDS (249-581)	Artificial - deimmunized light chain J591	Fig. 4B	25	aagcttatgaatatgcaaatcctctgaatctacatggttaatataggt ttgtctataccacaaacagaaaaacatgagatcacagttctcttac agttactgagcacacaggacctcaccatgggatggagctgtatcat cctctcttgtagcaacagctacaggttaaggggctcacagtagca ggcttgaggtctggacatatatatgggtgacaatgacatccacttg cctttctctccaggtgtccactccgacatccagatgaccagctct ccctcatccctgtccacatcagtaggagacaggggtcacccctcacct gtaaggccagtcaagatgtgggtactgtctgtagactggtatcaaca gaaaccaggacctctcctaaactactgatttattggcatccactc ggcacactggaatccctagtctgtcctcaggcagtggtatctggga cagacttcactctcaccatttctagtcttcagcctgaagacttgcag attattactgtcagcaatataacagctatcccttcacgttcggctctg ggaccaagggtggacatcaaacgtgagtagaatttaaaccttgcctc ctcagttggatcc
V _L Deimmunized (complimentary strand of SEQ ID NO:25) J591	Artificial - deimmunized light chain J591	Fig. 4B	26	ggatccaactgaggaagcaaagttaaattctactcacgtttgatgt ccacctgtgtccaggaccgaacgtgagaggatagctgttatattg ctgacagtaataatctgcaaagcttcaggctgaagactagaaatg gtgagagtgaagtctgtcccagatccactgcctgagaagcgacta gggattccaggtgtccgagtggtgacccaataaatcagtagtttag gagatgtcctgtgttctgttgataccagctacagcagtagccaca tcttgactggccttacaggtgagggtgacctgtctcctactgatgt

				ggacagggatgagggagactgggtcatctggatgtcggagtggacacctgtgagagaaaaggcaaagtggatgtcattgtcacccatataatgtccagacctcaagcctgtactgtgagcccctacctgtagctgtgctaccaagaagaggatgatacagctccatcccatggtaggtctctgtgtgctcagtaactgtagagagaactgtgatctcatgttttctgtttgtgtatagacaaacctatattaccatgtagattcagaggattgcatattcataagctt
V _H Deimmunized (predicted a.a. of SEQ ID NO:23) J591	Artificial - deimmunized heavy chain J591	Fig. 4A	27	MGWSCILFLVATATGVHSEVQLVQSGPE VKKPGATVKISCKTSGYTFTEYTIHWVKQ APGKGLEWIGNINPNNGGTTYNQKFEDKA TLTVDKSTDYAMELSSLRSEDTAVYYCA AGWNFDYWGGQGLTLTVSS
V _L Deimmunized (predicted a.a. of SEQ ID NO:25) J591	Artificial - deimmunized light chain J591	Fig. 4B	28	MGWSCILFLVATATGVHSDIQMTQSPSSL STSVGDRVTLTKASQDVGTAVDWYQQK PGPSPKLLIYCASTRHTGIPSRFSGSGSGTD FTLTSSLQPEDFADYYCQQYNSYPLTFGP GTKVDIK
V _H Variable Region J415	Mus musculus	Fig. 5	47	EVKLEESGGGLVQPGGSMKLSVASGFTF SNYWMNWVRQSPEKGLEWVAEIRSQSN FATHYAESVKGRVIISRDDSKSSVYLQMN NLRAEDTGIYYCTRRWNNFWGQGTTLTV SS
V _L Variable Region J415	Mus musculus	Fig. 6	48	NIVMTQFPKSMSSISVGERVTLTKASENV TYVSWYQQKPEQSPKMLIYGASNRFTGVP DRFTGSGSATDFILTISSVQTEDLVDYYCG QSYTFPYTFGGGKLEMK
V _H Variable Region (Deimm) J415-4	Artificial - deimmunized heavy chain J415-4	Fig. 5	49	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQSPEKGLEWVAEIRSQSNF ATHYAESVKGRVIISRDDSKSSVYLQMNS LRAEDTAVYYCTRRWNNFWGQGTITVTS S
V _L Variable Region (Deimm) J415-5	Artificial - deimmunized light chain J415-5	Fig. 6	50	NIVMTQFPKSMSSASAGERMTLTCKASENV GTIVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISSVQAEDLVDYYC QSYTFPYTFGGGKLEMK
V _H Deimmunized J415-4	Artificial - deimmunized heavy chain J415-4		51	gaagtgaacttgaggagtctggaggaggcttggtgcaacctggagggtccatgaaaatctcctgtgtgctccttggttcactttcagtaa ttactggatgaactgggtcgccagctccagagaaggggctga gtgggtgctgaaattagatcgcaatctaataatttgcacacattat gcggagtctgtgaaaggagggtcatcatctcaagagatgattcc aagagtgtgtctacgtcaaatgaacagttgagagctgaagaca ctgccgtttattactgtaccaggcgatggaataatttctggggccaa ggcaccactgtcacagctcctca
V _L Deimmunized	Artificial - deimmunized		52	aacattgtaatgacccaatttccaaatccatgtccgcctcagcagg agagaggatgacctgacctgcaaggccagtgagaatgtgggta

J415-5	light chain J415-5			cttatgtgtcctggtatcaacagaaaccaacacagtcctctaagatg ttgatatacggggcatccaacgggtcactgggtcccagatcgct tctccggcagtggtatctggaacagatttcattctgaccatcagcagt gtgcaggcagaagacctttagattattactgtggacagagttaca cctttccptacacgttcggaggggggaccaagctggaaatgaag
V _H Deimmunized J415-1 CDS (122- 160) & CDS (249- 608) Mature (18- 133)	Artificial - deimmunized heavy chain J415-1	Fig. 7A	53	aagcttatgaatatgcaaatcctctgaatctacatggtaaataaggt ttgtctataccacaacagaaaaacatgagatcacagttctcttac agttactgagcacacaggacctcaccatgggagtgagctgtatca tcctcttcttgtagcaacagctacaggttaagggtcacagtagc aggcttgaggtctggacatatatatgggtgacaatgacatccactt gctttctctcacaggtgtccactccgaagtgaacttgaggagt ctggaggaggtctgtgcaacctggagggtccatgaaaatctcct gtaaagcctctggattcattcagtaattactggatgaactgggtcc gccagactccagagaaggggcttgagtggtgtcttattagatc gcaatctaataattttgcaacacattatcgaggtctgtgaaaggga gggtcatcatctcaagagatgattccaagagtagtctacctgca aatgaacagtttgagagctgaagacactgccgtttattactgtacca ggcgtatggaataattttcggggcgaaggcaccactgtcacagct cctcaggtgagtccttacaacctctctcttattcagcttaaatagat tttactgcatittgtgggggggaaatgtgtgtatctgaatttcaggtca tgaaggactagggacacctgggagtcagaaagggtcattggga gcccgggctgatgcagacagacatcctcagctccagacttcag gccagagatttataggatcc
V _H Deimmunized (predicted a.a. of SEQ ID NO:53) J415-1	Artificial - deimmunized heavy chain J415-1	Fig. 7A	54	MGWSCILFLVATGVHSEVKLEESGGGLV QPGGSMKISKASGFTFSNYWMNWVRQT PEKGLEWVALIRSQSNFATHYAESVKGR VIISRDDSKSSVYLQMNSLRAEDTAVYYC TRRWNNFWGQGTITVTVSS
V _H Deimmunized (complimentar y strand of SEQ ID NO:53) J415-1	Artificial - deimmunized heavy chain J415-1	Fig. 7A	55	ggatcctataaatctctggccatgaagctctgggagctgaggatgtc tgtctgcatcagcccgggtcccaatgacctttctgactcccaag gtgtccctagtccttcagctgaaattcagatacacacatttcccc ccaacaatgcaagtaaaatctatttaagctgaatagaagagagag gttgaaggactcacctgaggagactgtgacagtgggtccttggc cccagaaattattccatcgctgtacagtaataaacggcagtgct tcagctctcaaaactgttcatttgaggtagacactacttggaaatca tctcttgagatgatgacctcccttcacagactccgcataatgtgtt gcaaaattattagattgcgatctaataagcaaccactcaagcc ccttctctggagctctggcgaccagttcatccagtaattactgaaa gtgaatccagagggtttacaggagattttcatggaccctccaggtg caccaagcctcctccagactcctcaagttcacttcggagtgagaca cctgtggagagaaaaggcaagtggtgtcattgtcacccatatata tgtccagacctcaagcctgctactgtgagccccttacctgtagctgt tgctaccaagaaggatgatacagctccatcccatgtgaggtc ctgtgtgctcagtaactgtagagagaactgtgatctcatgttttctgt ttgtggtatagacaaacctataattaccatgtagattcagaggatttg catattcataagctt
V _L Deimmunized	Artificial - deimmunized	Fig. 8A	56	aagcttatgaatatgcaaatcctctgaatctacatggtaaataaggt ttgtctataccacaacagaaaaacatgagatcacagttctcttac

J415-1 CDS (122-160) & CDS (249-581)	light chain J415-1			agttactgagcacacaggacctcaccatgggatggagctgtatca tcctctcttggttagcaacagctacaggtaaggggtcacagtagc aggcttgaggctctggacatatatatgggtgacaatgacatccattt gccttctctccacaggtgtccactccaacattgtaatgaccaatc cccaaatccatgtccgcctcagcaggagagaggatgacctgac ctgcaaggccagtgagaattccggtacttatgttcctggtatcaac agaaaccaacacagttctctaagatgttgatatacggggcatccaa ccggttcactgggggtccagatcgcttctccggcagtgatctgga acagatttacttctgaccgccagcagtggtgcaggcagaagaccct gtagattattactgtggacagagttacaccttccgtacacgttcgga gggggggaccaagctggaaatgaagcgtgagtagaatttaaactt gcttctcagttggatcc
V _L Deimmunized (predicted a.a. of SEQ ID NO:56) J415-1	Artificial - deimmunized light chain J415-1	Fig. 8A	57	MGWSCILFLVATGVHNSIVMTQSPKMS ASAGERMTLTCKASENSGTYVSWYQQK TQSPKMLIYGASNRFTGVPDRFSGSGSGTD FILTASSVQAEDPVDYYCGQSYTFPYTFGG GTKLEMK
V _L Deimmunized (complimentar y strand of SEQ ID NO:56) J415-1	Artificial - deimmunized light chain J415-1	Fig. 8A	58	ggatccaactgaggaagcaaagtttaattctactcacgcttcatttc cagcttggtcccccctccgaacgtgtacggaaggtgtaactctgt ccacagtaataatctacagggtcttctgcctgcacactgctggcgg tcagaatgaaatctgttccagatccactgccggagaagcagatctgg gacccagtgaaacgggtggatgcccgatatcaacatcttagga gactgtgttggttctgttgataccaggacacataagtaaccggaatt ctcactggccttgcaaggtcaaggtcatcctctctcctgctgaggcg gacatggatttggggattgggtcattacaatgttgagtgacac ctgtggagagaaaggcaaagtggatgtcattgtcacccatatatat gtccagacctcaagcctgctactgtgagccccctacctgtagctgtt gctaccaagaagaggatgatacagctccatcccatggtgaggtcc tgtgtgctcagtaactgtagagagaactgtatctcatgttttctgtt gtggtatagacaaacctatattaccatgtagattcagaggatttgc tattcataagctt
V _H Deimmunized J415-2	Artificial - deimmunized heavy chain J415-2	Fig. 5	59	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVALIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMN LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
V _H Deimmunized J415-3	Artificial - deimmunized heavy chain J415-3	Fig. 5	60	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVAEIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMN LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
J415 V _H (DI) majority sequence	Artificial - majority sequence	Fig. 5	61	EVKLEESGGGLVQPGGSMKISCVASGFTFS NYWMNWVRQTPEKGLEWVAEIRSQSNNF ATHYAESVKGRVIISRDDSKSSVYLQMN LRAEDTAVYYCTRRWNNFWGQGTTVTVS S
V _L Deimmunized J415-2	Artificial - deimmunized light chain	Fig. 6	62	NIVMTQSPKMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTASSVQAEDPVDYYC

	J415-2			GQSYTFPYTFGGGKLEMK
V _L Deimmunized J415-3	Artificial - deimmunized light chain J415-3	Fig. 6	63	NIVMTQSPKMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTASSVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
V _L Deimmunized J415-4	Artificial - deimmunized light chain J415-4	Fig. 6	64	NIVMTQSPKMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
V _L Deimmunized J415-6	Artificial - deimmunized light chain J415-6	Fig. 6	65	NIVMTQFPKMSASAGERMTLTCKASENV GTYVSWYQQKPEQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
V _L Deimmunized J415-7	Artificial - deimmunized light chain J415-7	Fig. 6	66	NIVMTQFPKMSASAGERVTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
V _L Deimmunized J415-8	Artificial - deimmunized light chain J415-8	Fig. 6	67	NIVMTQFPKMSASAGERMTLTCKASENS GTYVSWYQQKPEQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
J415 V _L (DI) majority sequence	Artificial - majority sequence	Fig. 6	68	NIVMTQFPKMSASAGERMTLTCKASENV GTYVSWYQQKPTQSPKMLIYGASNRFTGV PDRFSGSGSGTDFILTISVQAEDLVDYYC GQSYTFPYTFGGGKLEMK
MuV _H IIC	Mus musculus	Fig. 7C	69	EVKLEESGGGLVQPGGSMKLSVASGFTF SNYWMNWVRQSPEKLEWVAEIRLKSDN YATHAESVKGRFTISRDDSKSSVYLQMN NLRAEDTGIYYCTTGGYGRRSWFAYWG QGTLTVSS
J415V _H /MuV H IIC majority sequence	Artificial - majority sequence	Fig. 7C	70	EVKLEESGGGLVQPGGSMKLSVASGFTF SNYWMNWVRQSPEKLEWVAEIRLQSDN FATHAESVKGRVIISRDDSKSSVYLQMN NLRAEDTGIYYCTTGGYGRRSWNAFWG QGTLTVSS
MuV _L 1	Mus musculus	Fig. 8C	71	DIVMTQSPSSLAVSAGEKVTMSCKSSQSL NSGNQKNYLAWYQQKPGQSPKLLIYAS TRESGVPDRFTGSGSGTDFLTISVQAED LAVYYCQNDYSYPLTFGAGTKLELK
J415V _L /MuV L1 majority sequence	Artificial - majority sequence	Fig. 8C	72	DIVMTQSPSSLAVSAGEKVTLSCKASESL NVGNQKTYVAWYQQKPGQSPKLLIYGAS TRESGVPDRFTGSGSGTDFLTISVQAEDL AVYYCGNSYSFPLTFGGGKLELK
J533 V _H CDS (1-354)	Mus musculus	Fig. 9A	73	gaggtccagctgcagcagctctggacctgagctggtaagcctggg gcttcagtggaagatgtcctgcaaggctctggatacacattcactgg ctatgttatgcactgggtgaagcagaagcctggacaggtccttgag tggtatggatatataatccttacaatgatgttactaggtataatggga agttcaaaggcaaggccacactgacctcagacaaatattcagca cagcctacatggagctcagcgccctgacctctgaggactctgcgg tctattactgtgcaagaggaggagaactgtactactttgactcctgg

				ggccgaggcgccactctcacagtctcctca
J533 V _H (predicted amino acid of SEQ ID NO:73)	Mus musculus	Fig. 9A	74	EVQLQQSGPELVKPGASVKMSCKASGYTF TGYVMHWVKQKPGQVLEWIGYINPYNDV TRYNGKFKGKATLTSDKYSSTAYMELSGL TSEDSAVYYCARGENWYYFDSWGRGATL TVSS
J533 V _H (complementar y strand of SEQ ID NO:73)	Mus musculus	Fig. 9A	75	tgaggagactgtgagagtggcgccctcgccccaggagtcaaaagt agtaccagttctcccctcttgacagtaatagaccgcagagtcctc agaggtcaggccgctgagctccatgtaggctgtgctggaatatttg tctgaggtcaggtggccttgctttgaacttccattatacctagta acatcattgtaaggattaatatccaatccactcaaggacctgtcc aggcttctgctcaccagtgataacatagccagtgatgtgtatc cagaagccttgaggacatcttactgaagccccaggcttaacca gctcagggtccagactgtgagctggacctc
J533 V _L CDS (1-333)	Mus musculus	Fig. 10A	76	gacattgtgctgacccaatctccagcttcttggtgtgtctctagga cagaggggccaccatctcgcagagccagtgaaagtattgatagt atgacaatactttatgcactggtaccagcagaaccaggacagcc acccaacctctcatcttctgctcatccatcctagaatctgggatcc ctgacaggttcagtgagcagtggtctgggacagacttcacctca ccattatctctgaggagctgatgatgtgcaacctattactgtcacc aaagtattgaggatcgtacacgttcggaggggggaccaagctg gaaataaaa
J533 V _L (predicted amino acid of SEQ ID NO:76)	Mus musculus	Fig. 10A	77	DIVLTQSPASLAVSLGQRATISCRASEIDS YDNTFMHWYQQKPGQPPNLLIFRAILES GIPARFSGSGSGTDFLTITYPVEADDVATY YCHQSIEDPYTFGGGKLEIK
J533 V _L (complementar y strand of SEQ ID NO:76)	Mus musculus	Fig. 10A	78	tttatttccagcttggtcccccctccgaacgtgtacggatcctcaat actttggtgacagtaaatagggtgcaacatcatcagcctccacaggat aaatgggtgagggtgaagtctgtccagaccactgccactgaacc tgaggggatccagattctaggatggatgcacgaagatgagg agggtgggtggtgctgctgtgttctgctgttaccagtgataaaagt attgtcataactatcaatacttctactggctctgaggatattgtggc cctctgtcctagagacacagccaagaagctggagattgggtcag cacaatgct
MuV _H II	Mus musculus	Fig. 9B	79	EVQLQQSGPELVKPGASVKISCKASGYTFT DYVMNWNWKQSPGKSLEWIGDINPGNGG TSYNQKFKGKATLTVDKSSSTAYMQLSSL TSEDSAVYYCARGYYSSSYMAYYAFDYW GQGTTVTVSS
J533V _H /MuV _H II majority sequence	Artificial - majority sequence	Fig. 9B	80	EVQLQQSGPELVKPGASVKISCKASGYTFT GYVMNWNWKQSPGQVLEWIGDINPGNGG TSYNGKFKGKATLTVDKSSSTAYMELSGL TSEDSAVYYCARGENSSSYMAYYAFDSW GQGATVTVSS
MuV _L -3	Mus musculus	Fig. 10B	81	DIVLTQSPASLAVSLGQRATISCRASESVDS YGNFSFMHWYQQKPGQPPKLLIYAASNL GVPARFSGSGSGTDFLTNIHPVEEDDAATY YCQQSNEDPPWTFGGGKLEIK
J533V _L /	Artificial -	Fig.	82	DIVLTQSPASLAVSLGQRATISCRASESVDS

MuV_L-3 majority sequence	majority sequence	10B		YGNSFMHWYQQKPGQPPNLLIFAASILES GVPARFSGSGSGTDFTLTIHPVEADDAATY YCQQSIEDPPYTFGGGTKLEIK
E99 V_H CDS (1-363)	Mus musculus	Fig. 11A	83	cagggtgcagctaaaggagtcaggacctggcctggctggcgtcctc acagagcctgtccatcacatgcaccgtctcaggattctcattaacc gcctatggtattaaactgggttcgccacctccaggaaagggtctgg agtggctgggagtgatatggcctgatggaaacacagactataattc aactctcaaataccagactgaacatctcaaggacaactccaagaac caagttttctaaaaatgagcagtttccaaactgatgacacagccag atactctgtgccagagattcgtatggtaactcaagagggttggtt tgacttctggggccaggggcaccactctcacagctctcctca
E99 V_H (predicted amino acid of SEQ ID NO:83)	Mus musculus	Fig. 11A	84	QVQLKESGPGLVASSQSLTITVSGFSLT AYGINWVRQPPGKGLEWLGVIPDGNTD YNSTLKSRLNIFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDWFGQGT TLTVSS
E99 V_H (complementar y strand of SEQ ID NO:83)	Mus musculus	Fig. 11A	85	tgaggagactgtgagagtggtgccctggccccagaagtc aaacc aaccctcttgaagttaccatacgaatctctggcacagaagtatctg gctgtgtcatcagtttgaaactgctcattttaagaaaactgggtctt ggagttgtcctgaagatgttcagttctggattgagagttgaattata gtctgtgttccatcaggccatatcactcccagccactccagacct ttcttgagggtggcgaaccagttaataccataggcggtaataga gaatcctgagacgggtgcatgtgatggacaggctctgtgaggacgc caccaggccaggctcctgactccttagctgcacctg
E99 V_L CDS (1-321)	Mus musculus	Fig. 12A	86	aacattgtgatgaccagtcctcaaaaattcatgtccacatcaccagg agacagggtcagggtcacctgcaaggccagtcagaatgtgggtt ctgatgtagcctgtgatcaagcgaaaccaggacaatctcctagaat actgatttactcgacatcctaccgttacagtggggtccctgatcgctt cacagcctatggatctggagacagatttactctcaccattaccaatg tgcagctgaagactgacagagtatttctgtcagcaatataatagct atcctctcacgttcggtgctgggaccaagctggagctgaaa
E99 V_L (predicted amino acid of SEQ ID NO:86)	Mus musculus	Fig. 12A	87	NIVMTQSQKFMSTSPGDRVRVTCKASQNV GSDVAWYQAKPGQSPRILIYSTSYRYSQV DRFTAYGSGTDFLTITNVQSEDLTEYFCQ QYNSYPLTFGAGTKLELK
E99 V_L (complementar y strand of SEQ ID NO:86)	Mus musculus	Fig. 12A	88	tttcagctccagcttggtcccagcaccgaacgtgagaggatagcta ttatattgctgacagaaatactctgtcaagttctcagactgcacattg gtaattggtgagagtgaaatctgtcccagatccataggctgtgaagc gatcaggggacccactgtaacggtaggatgtcgagtaaatcagta ttctaggagattgtcctggttcgcttgataccaggctacatcagaac ccacattctgactggccttgagggtgacctgacctgtctcctggt gatgtggacatgaattttgagactgggtcatcacaatgtt
MuV_HIB	Mus musculus	Fig. 11B	89	QVQLKESGPGLVASSQSLTITVSGFSLT AYGINWVRQPPGKGLEWLGVIPDGNTD YNSTLKSRLNIFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDWFGQGT TLTVSS
E99V_H/MuV_H IB	Artificial - majority	Fig. 11B	90	QVQLKESGPGLVASSQSLTITVSGFSLT AYGINWVRQPPGKGLEWLGVIPDGNTD

majority sequence	sequence			YNSTLKSRNLNFKDNSKNQVFLKMSSFQT DDTARYFCARDSYGNFKRGWFDWGGQT TLTVSS
MuV _L -1	Mus musculus	Fig. 12B	91	DIVMTQSPSSLAVSAGEKVTMSCKSSQSL NSGNQKNYLAWYQQKPGQSPKLLIYWAS TRESGVPDRFTGSGSGTDFTLTISVQAED LAVYYCQNDYSYPLTFGAGTKLELK
E99V _L /MuV _L -1 majority sequence	Artificial - majority sequence	Fig. 12B	92	DIVMTQSQSSLAVSAGDKVTVSCKASQSL LNVGSDKNYVAWYQAKPGQSPKLLIYSAS TRESGVPDRFTGSGSGTDFTLTISVQAED LAVYFCQNDNSYPLTFGAGTKLELKRA

[00151] Humanized or CDR-grafted antibody molecules or immunoglobulins can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDRs of an immunoglobulin chain can be replaced. See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference.

[00152] Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

[00153] Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a predetermined target, as described above. The recombinant DNA encoding

the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

[00154] Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., US 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. The acceptor framework can be a mature human antibody framework sequence or a consensus sequence.

[00155] As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

[00156] Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

[00157] The anti-PSMA antibody, or antigen fragment thereof, may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317, the contents of which are specifically incorporated by reference herein. Briefly, the murine heavy and light chain variable regions of an anti-PSMA antibody can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modelling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the murine V_H and V_L sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T

cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible conservative substitutions are made, often but not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in Tomlinson, I.A. *et al.* (1992) *J. Mol. Biol.* 227:776-798; Cook, G. P. *et al.* (1995) *Immunol. Today* Vol. 16 (5): 237-242; Chothia, D. *et al.* (1992) *J. Mol. Bio.* 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I.A. *et al.* MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunized V_H and V_L of an anti-PSMA antibody are constructed by mutagenesis of the murine V_H and V_L genes. The mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or κ constant regions.

[00158] In some cases a potential T cell epitope will include residues which are known or predicted to be important for antibody function. For example, potential T cell epitopes are usually biased towards the CDRs. In addition, potential T cell epitopes can occur in framework residues important for antibody structure and binding. Changes to eliminate these potential epitopes will in some cases require more scrutiny, e.g., by making and testing chains with and without the change. Where possible, potential T cell epitopes that overlap the CDRs were eliminated by substitutions outside the CDRs. In some cases, an alteration within a CDR is the only option, and thus variants with and without this substitution should be tested. In other cases, the substitution required to remove a potential T cell epitope is at a residue position within the framework that might be critical for antibody binding. In these cases, variants with and without this substitution should be tested. Thus, in some cases several variant deimmunized heavy and light chain variable regions were designed and various heavy/light chain combinations tested in order to identify the optimal deimmunized antibody. The choice of the final deimmunized antibody can then be made by considering the binding affinity of the different variants in conjunction with the extent of deimmunization, i.e., the number of potential T cell epitopes remaining in the variable region.

[00159] The recombinant deimmunized antibody can be transfected into a suitable host cell for expression, for example, NS0 or CHO cells, to produce complete recombinant antibodies.

[00160] In one embodiment, deimmunized V_H and V_L of murine J591 regions were constructed by mutagenesis of the murine V_H and V_L genes. The murine J591 variable region sequences are shown in Figures 1A-1B. Potential epitopes (identified using a peptide threading program) in murine J591 heavy chain and light chain variable regions are shown in Figures 3A and 3B, respectively. The 13-mer peptides predicted to bind to MHC class II are indicated by the underline, the CDRs are located at residues 26 to 35, 50 to 66, and 99 to 104 of Figure 3A and residues 24 to 34, 50 to 56, and 89 to 97 of Figure 3B, and residues altered in the deimmunized heavy and light chain variable regions are boxed. Where possible, amino acid substitutions are those commonly used in human germline heavy and light chain variable regions. In addition to the *in silico* analysis using the peptide threading software, a database of human MHC class II binding peptides was searched for motifs present in the murine J591 sequence.

[00161] The following 13-mers (labelled by first linear residue number of the 13-mer) of the murine J591 heavy chain variable region were predicted to bind to MHC Class II were 2, 10, 16, 30, 32, 35, 43, 46, 58, 62, 70, 81, 84, 91, and 100 (Figure 3A). An explanation of the rationale behind changes made to the residues in the murine J591 heavy chain variable region is set forth below (note residues altered are identified under the Kabat numbering system):

5Q→V removes the potential epitope at residue 2;
 11,12LV→VK remove the potential epitope at residue 10;
 12V→K is also changed to remove a motif from the database of human MHC class II binding peptides;
 16,17TS→AT, and 19R→K remove the potential epitope at residue 16;
 the epitope at residue 30 spans CDR1 and is therefore unaltered;
 40,41SH→AP removes potential epitopes at residues 32 and 35;
 44S→G reduces binding score for epitope at 43, this 13 mer spans CDR2;
 the epitopes at residues 46, 58 and 62 span CDR2, and are thus unaltered;
 75,76SS→TD remove the potential epitope at residue 70;
 82aR→S, 83T→R remove potential epitopes at residues 81 and 84;
 87S→T this change made to remove a motif from the database of human MHC class II binding peptides;
 the epitope at residue 91 spans CDR3 and is therefore unaltered; and

108T→L removes the potential epitope at residue 100.

[00162] The following 13-mers (labelled by first linear residue number of the 13-mer) of the murine J591 light chain variable region that were predicted to bind to MHC Class II molecules were 1, 8, 17, 27, 30, 31, 35, 45, 47, 56, 60, 71, 73, 81, 94 (Figure 3B). An explanation of the rationale behind changes made to the residues in the murine J591 light chain variable region is set forth below (note residues altered are identified under the Kabat numbering system):

3V→Q removes potential epitope at residue 1;

8-11HKFM→PSSL removes potential epitope at residue 8(13);

20-22SII→TLT removes potential epitopes at residues 17 and 20;

21I→L is also changed to remove a motif from the database of human MHC class II binding peptides;

the epitope at residue 27 spans CDR1 and is therefore unaltered;

42Q→P reduces the binding score for the epitope at residue 31;

the epitopes at residues 44 and 47 span CDR2 and are thus unaltered;

58V→I is changed to remove a motif from the database of human MHC class II binding peptides;

60D→S, 62T→S removes the epitopes at residues 56 and 60;

76-78TNV→SSL, 80S→P, 83L→F removes the epitopes at residues 71, 73, 76, and 81;

87F→Y I is changed to remove a motif from the database of human MHC class II binding peptides;

100 A→P and 103 M→K remove the epitope at residue 94; and

104 L→V and 106 L→I are changed to remove a motif from the database of human MHC class II binding peptides.

[00163] The amino acid and nucleotide sequences for the deimmunized J591 heavy and light chain variable regions are shown in Figures 2A-2B and 4A-4B, respectively (see also Table 8).

[00164] Human IgG1 or κ constant regions were added and the composite genes transfected into NS0 cells to produce complete recombinant anti-PSMA antibodies. These

antibodies bound to PSMA (on LNCap cells) as efficiently as the original murine antibody, and have reduced or no immunogenicity in man.

[00165] The design of deimmunized J415 was similar to the making of the deimmunized J591 antibody. The heavy and light chain sequences were cloned from the hybridoma designated HB-12109. These sequences were cloned, sequenced and expressed as a chimaeric antibody for use as a control antibody. The murine V region sequences were subjected to peptide threading to identify potential T cell epitopes, through analysis of binding to 18 different human MHC class II allotypes. The results of the peptide threading analysis for the murine sequences are shown in Table 9.

Table 9: Potential T cell epitopes in murine J415 sequences

Sequence	Number of potential T cell	Location of potential epitopes+ (no. of potential MHC binders from 18 groups tested)
Murine J415 V _H	12	10(17), 16(13), 21(9), 30(6), 35(16), 43(8), 46(6), 49(8), 64(6), 80(15), 86(15), 104(6)
Murine J415 V _K	13	5(5), 11(18), 13(11), 17(5), 27(8), 31(7), 56(15), 60(12), 70(5), 71(11), 73(17), 76(7), 81(17)

*first amino acid of potential epitope, numbering E or N amino acid number 1 to S or K amino acid number 107 and 116 for V_H and V_K respectively.

[00166] Primary deimmunized V_H and V_L sequences were defined (J415DIVH1, J415DIVK1). As generation of the primary deimmunized sequences requires a small number of amino acid substitutions that might affect the binding of the final deimmunized molecule, three other variant V_HS and seven other V_LS were designed (see Figures 5 and 6). The nucleotide sequences for the primary deimmunized V_H and V_L regions are shown in Figures 7A and 8A, respectively. Comparisons of the amino acid sequences of the murine and deimmunized V regions of J415 are shown in Figure 5 for V_H and Figure 6 for V_L.

[00167] An explanation of the rationale behind some of the changes made to the residues in the murine J415 heavy chain variable region is set forth below (note residues altered are identified according to the linear numbering shown in Figure 5):

20L→I removes the potential epitope at residues 10 and 16;

87N→S removes the potential epitopes at residues 80 and 86;

94,95GI→AV remove the potential epitope at residue 86; and

112L→V removes the potential epitope at residue 104.

[00168] An explanation of the rationale behind some of the changes made to the residues in the murine J415 light chain variable region is set forth below (note residues altered are identified according to the linear numbering shown in Figure 6):

13I A removes the potential epitopes at residues 5, 11 and 13;

15V A removes the potential epitopes at residues 5, 11, and 13;

19V-M removes the potential epitopes at residues 11, 13, and 17;

41E-T removes the potential epitope at residue 31;

63T-S removes the potential epitopes at residues 56 and 60;

68A-G removes the potential epitopes at residues 56 and 60; and

80T-A removes the potential epitopes at residues 70, 71, 73, and 76;

[00169] The deimmunized variable regions for J415 were constructed by the method of overlapping PCR recombination. The cloned murine V_H and V_K genes were used as templates for mutagenesis of the framework regions to the required deimmunized sequences. Sets of mutagenic primer pairs were synthesized encompassing the regions to be altered. The vectors V_H-PCR1 and V_K-PCR1 (Riechmann *et al.* (1988) *Nature* 332:323-7) were used as templates to introduce 5' flanking sequence including the leader signal peptide, leader intron and the murine immunoglobulin promoter, and 3' flanking sequence including the splice site, and intron sequences. The deimmunized V regions produced were cloned into pUC19 and the entire DNA sequence was confirmed to be correct for each deimmunized V_H and V_L.

[00170] The deimmunized heavy and light chain V-region genes were excised from pUC19 as *Hind*III to *Bam*HI fragments, which include the murine heavy chain immunoglobulin promoter, the leader signal peptide, leader intron, the V_H or V_L sequence and the splice site. These were transferred to the expression vectors pSVgpt and pSVhyg, which include human IgG1 or κ constant regions respectively and markers for selection in mammalian cells. The DNA sequence was confirmed to be correct for the deimmunized V_H and V_L in the expression vectors.

[00171] For the transfection of expression vectors pSVgptJ415V_HHuIgG1 and pSVhygJ415V_KHuCK into NS0 (a non-immunoglobulin producing mouse myeloma, obtained from the European Collection of Animal Cell Cultures, Porton UK (ECACC No. 85110503)) cells, 3 and 6 ug of plasmid DNA respectively was prepared and then linearized with *Pvu*I to

improve transfection efficiency. The ethanol precipitated DNA was then mixed with a semi-confluent flask of NS0 cells that had been harvested by centrifugation and resuspended in 0.5 ml of non-selective Dulbecco's Modified Eagle's Medium (DMEM)(Life Technologies Inc.) in a 0.4 cm gene pulser cuvette. The cells and DNA were chilled on ice for 5 minutes before a pulse of 170V, 960 μ F was applied. The cuvette was returned to ice for a further 20 minutes before being transferred to a 75cm² flask containing 20mls non-selective DMEM to recover for 24 hours. The cells were then harvested and resuspended in selective DMEM and plated over 4x96 well plates, 200 μ l/well. A similar protocol was followed for the transfection of expression vectors encoding the deJ591 antibody heavy chain and light chain subunits into NS0 cells.

[00172] To culture, select, and expand NS0 cell lines, the cells are grown at 37°C, 5%CO₂ and 10% FBS. To prepare non-selective medium for routine culture of NS0 cells, the culture medium is Dulbecco's Modification of Eagle's Medium (DMEM)(Life Technologies, Catalogue No: 31965-023) supplemented with 10% fetal bovine serum of USA origin (Life Technologies, Fetal Bovine Serum Cat No: 16000), Antibiotic/Antimycotic solution (Life Technologies, Cat No: 15240), Gentamycin (Life Technologies, catalogue No: 15710), Sodium pyruvate (Life Technologies, Catalogue No: 11360-039). When growing NS0 cells up to saturation for antibody production do not add the xanthine and mycophenolic acid and the FBS is reduced to 5%.

[00173] To prepare selective medium for culture of NS0 transfectomas, the culture medium is Dulbecco's Modification of Eagle's Medium (DMEM)(Life Technologies, Catalogue No: 31965-023) supplemented with 10% foetal bovine serum of USA origin (Life Technologies, Fetal Bovine Serum Cat No: 16000), Antibiotic/Antimycotic solution (Life Technologies, Cat No: 15240), Gentamycin (Life Technologies, catalogue No: 15710), Sodium pyruvate (Life Technologies, Catalogue No: 11360-039), 250 μ g/ml xanthine (Sigma Catalogue No: X-3627, stock made up at 25 mg/ml in 0.5M NaOH), and 0.8 μ g/ml mycophenolic acid (Sigma Catalogue No: M-3536, stock made up at 2.5 mg/ml in 100% ethanol).

[00174] After approximately 10 days the cell colonies expressing the *gpt* gene were visible to the naked eye. The plates were then screened for antibody production using the following protocol for human IgG1/ κ Screening ELISA. 6 single colonies were picked from wells with high ODs greater than 0.7 into a 24 well cell culture plate. Within 5-6 days the cells were expanded into a 25cm² flask. The antibody productivity of the selected clones was assayed using

the following protocol for human IgG1/ κ ELISA from saturated cultures in the 24 well and 25cm² flasks.

[00175] The details of the protocol are as follows. ELISA plates (Dynatech Immulon 2) are coated with 100 μ L per well with sheep α human κ antibody (The Binding Site Cat No: AU015) diluted 1:1000 in carbonate/bicarbonate coating buffer pH9.6 (Sigma Cat: C-3041). The coated plate is incubated at 4°C overnight or 1 hr at 37°C. The plate is then washed 3 times with PBST (PBS with 0.05% Tween 20). The samples are added, 100 μ L per well from 24 well plates, 25 μ L + 75 μ L PBST for 96 well plates. Blank wells are treated with PBST only. The reaction mixture is incubated at room temperature for 1 hr. Then, the plate is wash 3 times with PBST (PBS with 0.05% Tween 20). The secondary antibody, peroxidase conjugated sheep α human IgG γ chain specific is added (The Binding Site Cat No: APO04) at a ratio of 1:1000 in PBST, 100 μ L per well. The mixture is incubated at room temperature for 1 hour. The mixture is then washed 3 times with PBST (PBS with 0.05% Tween 20).

[00176] To make up the substrate, one tablet (20 mg) of OPD (o-PHENYLENE DIAMINE) (Sigma Cat No: P-7288) is dissolved in 45 ml of H₂O plus 5ml 10 x peroxidase buffer (make 10 x peroxidase buffer with Sigma phosphate citrate buffer tablets pH 5.0, P-4809), add 10 μ L 30%(w/w) hydrogen peroxide (Sigma Cat No: H1109) just before use. The substrate is then added at 100 μ L per well and incubate RT for 5 min or as required. When the color develops, the reaction can be stopped by adding 25 μ L 12.5% H₂SO₄. The results are read at 492 nm.

Expression and expansion of J591 and J415 Deimmunized Antibodies

[00177] The clones with the highest productivity were expanded into a 75 cm² flask and then into 2x 175 cm² flasks. The cells from one of the 175 cm² flask was used to inoculate 4x triple layer flasks (500 cm², Nalge Nunc International) containing non selective DMEM containing 5% FBS, cells from the other were frozen as detailed in the protocol for freezing NS0 cells detailed below.

[00178] To cryoprotect mammalian cells and resurrect cells from liquid nitrogen, the following materials are needed: Fetal Bovine serum (Life Technologies Cat No: 16000), DMSO (Sigma, Cat No: D4540), 2 ml cryotubes (Nunc or Greiner), and polystyrene box with walls 1 - 2

cm thick. Briefly, actively growing cells are harvested by centrifugation (1000 rpm, 5 min) and resuspended at about 10^7 cells/ml in 10% DMSO/90% FBS. As a rough guide, cells grown to a semi-confluency should be resuspended in 1 ml for a 75 cm² flask or 2.5 ml for a 175 cm² flask. A required number of tubes are cooled and labeled in ice. 1 ml portions are aliquoted to labeled cryotubes. The cryotubes are placed in polystyrene box at -70°C for at least 4 h, or overnight. The vials are transferred to canes and place in liquid nitrogen. A record of the storage should be made both in the canister index and the central cell line indexing system.

[00179] To thaw the cells from liquid nitrogen, the vial is removed from liquid nitrogen and contents are thawed quickly by incubation at 37°C, while swirling in a waterbath. The outside of the vial is cleaned with 70% methylated spirits. The suspension is transferred to a universal container. 10 ml of the medium to be used to propagate the cell line is added dropwise, swirling to mix. The cells are harvested by centrifugation (1000 rpm, 5 min). The supernatant is discarded. The cells are resuspended in 20 ml growth medium and transfer to a 75 cm² flask. If low viability is suspected, extra serum can be added to the growth medium to 20%, use only 5 ml, and transfer to a 25 cm² flask.

[00180] After 10-14 days the 500 ml to 1 liter static saturated cultures were harvested. Antibody was purified, by ProSepA (Millipore Ltd.) affinity chromatography using the following protocol for antibody purification. The purified antibody preparation was sterilized by filtration and stored at 4°C.

[00181] The antibody purification protocol is as follows: NS0 transfectoma cell line producing antibody is grown in DMEM 5% FCS in Nunc Triple layer flasks, 250 ml per flask (total volume 1L) for 10 - 14 days until nearing saturation. Conditioned medium collected and spun at 3000 rpm for 5 min in bench centrifuge 5 mins to remove cells. 1/10th volume 1M Tris-HCl pH 8 (Sigma Cat: T3038) is then added to cell supernatant to make this 0.1 M Tris-HCl pH8. 0.5 to 1 ml Prosep A (Millipore Cat: 113 111824) is added and stirred overnight at room temperature. Prosep A collected by spinning at 3000 rpm for 5 mins then packed into a Biorad Poly-Prep column (Cat: 73 1-1550). The column is washed with 10ml PBS, then eluted in 1 ml fractions with 0.1M Glycine pH 3.0. Each fraction is collected into a tube containing 100 microL 1M Tris-HCl pH 8 (Sigma, as above). Absorbance of each fraction is measured at 280 nm. Fractions containing the antibody are pooled and dialyzed against PBS overnight at room temperature. The preparation is sterilized by filtration through a 0.2 micron syringe filter and the

absorbance of each fraction is measured at 280nm. The antibody concentration is determined by ELISA for human IgG.

[00182] The purified antibody can be quantified using the protocol for Human IgG/ κ ELISA described above.

Testing of J415 Deimmunized antibodies

[00183] The J415 deimmunized antibodies (including various combinations of the deimmunized light chain and heavy chain subunits) were tested in an ELISA for binding to LNCap membrane preparation following the protocol as detailed above. ELISA plates were coated with LNCap membrane preparation and blocked with 5% BSA in phosphate buffered saline. Doubling dilutions of the J415 chimeric antibody (murine variable heavy and light chain regions fused to human constant heavy and light chain regions, respectively) and the deimmunized antibodies were applied. Detection was with horseradish peroxidase conjugated goat anti-human IgG and donkey anti-mouse for chimeric and mouse antibodies respectively. Color was developed with o-phenylene diamine substrate.

[00184] The antibody composed of deimmunized J415 heavy chain version 4 combined with deimmunized J415 light chain version 5 shows equivalent binding to LNCap cells as compared to the chimeric antibody. Also, when DIVK5 is combined with heavy chain versions 1 and 2, binding to LNCap cells is equivalent to that of the chimeric antibody when tissue culture supernatant is analyzed. These data can be confirmed with purified antibody. When light chains 1, 2, 3 were combined with any of the J415 heavy chain versions 1, 2, 3, and 4 no antibody was produced. Deimmunized J415 light chain versions 1, 2, and 3 may be defective on structural grounds. The best chain combination for higher affinity and decreased immunogenicity is DIVH4/DIVK5.

[00185] The antibody composed of deimmunized heavy chain version 4 combined with deimmunized light chain version 5 showed equivalent binding to LNCap compared to the chimeric antibody. Also, when DIVK5 is combined with heavy chain versions 1 and 2, binding to LNCap cells is two-fold less than that of the chimeric when purified antibody is analyzed.

[00186] Monoclonal anti-PSMA antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology.

[00187] Anti-PSMA antibodies that are not intact antibodies are also useful in this invention. Such antibodies may be derived from any of the antibodies described above. For example, antigen-binding fragments, as well as full-length monomeric, dimeric or trimeric polypeptides derived from the above-described antibodies are themselves useful. Useful antibody homologs of this type include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR), e.g., one or more isolated CDRs together with sufficient framework to provide an antigen binding fragment. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[00188] Antibody fragments may also be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, and optionally treating the cleaved product with a reducing agent. Alternatively, useful fragments may be produced by using host cells transformed with truncated heavy and/or light chain genes.

[00189] Monoclonal, chimeric, humanized, deimmunized antibodies, which have been modified by, e.g., deleting, adding, or substituting other portions of the antibody, e.g., the constant region, are also within the scope of the invention. For example, an antibody can be modified as follows: (i) by deleting the constant region; (ii) by replacing the constant region with another constant region, e.g., a constant region meant to increase half-life, stability or affinity of the antibody, or a constant region from another species or antibody class; or (iii) by modifying one or more amino acids in the constant region to alter, for example, the number of

glycosylation sites, effector cell function, Fc receptor (FcR) binding, complement fixation, among others.

[00190] In one embodiment, the constant region of the antibody can be replaced by another constant region from, e.g., a different species. This replacement can be carried out using molecular biology techniques. For example, the nucleic acid encoding the VL or VH region of a antibody can be converted to a full-length light or heavy chain gene, respectively, by operatively linking the VH or VL-encoding nucleic acid to another nucleic acid encoding the light or heavy chain constant regions. The sequences of human light and heavy chain constant region genes are known in the art. Preferably, the constant region is human, but constant species from other species, e.g., rodent (e.g., mouse or rat), primate, camel, rabbit can also be used. Constant regions from these species are known in the art (see e.g., Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[00191] Methods for altering an antibody constant region are known in the art. Antibodies with altered function, e.g. altered affinity for an effector ligand, such as FcR on a cell, or the C1 component of complement can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (see e.g., EP 388,151 A1, US 5,624,821 and US 5,648,260, the contents of all of which are hereby incorporated by reference). Similar type of alterations could be described which if applied to the murine, or other species immunoglobulin would reduce or eliminate these functions.

[00192] An anti-PSMA antibody, or antigen-binding fragment thereof, can be derivatized or linked to another functional molecule (e.g., another peptide or protein). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the antibodies described herein, including immunoadhesion molecules. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[00193] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

[00194] Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized (or labeled) to include fluorescent compounds, various enzymes, prosthetic groups, luminescent materials, bioluminescent materials, fluorescent emitting metal atoms, *e.g.*, europium (Eu), and other anthanides, and radioactive materials (described below). Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, β -galactosidase, acetylcholinesterase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with a prosthetic group (*e.g.*, streptavidin/biotin and avidin/biotin). For example, an antibody may be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of bioluminescent materials include luciferase, luciferin, and aequorin.

[00195] Labeled antibodies can be used, for example, diagnostically and/or experimentally in a number of contexts, including (i) to isolate a predetermined antigen by standard techniques, such as affinity chromatography or immunoprecipitation; (ii) to detect a predetermined antigen (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein; (iii) to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen.

[00196] An anti-PSMA antibody or antigen-binding fragment thereof may be conjugated to a another molecular entity, typically a label or a therapeutic (e.g., a cytotoxic or cytostatic) agent or moiety.

[00197] Radioactive isotopes can be used in diagnostic or therapeutic applications. Radioactive isotopes that can be coupled to the anti-PSMA antibodies include, but are not limited to α -, β -, or γ -emitters, or β - and γ -emitters. Such radioactive isotopes include, but are not limited to iodine (^{131}I or ^{125}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), rhodium (^{188}Rh), sulfur (^{35}S), carbon (^{14}C), tritium (^3H), chromium (^{51}Cr), chlorine (^{36}Cl), cobalt (^{57}Co or ^{58}Co), iron (^{59}Fe), selenium (^{75}Se), or gallium (^{67}Ga). Radioisotopes useful as therapeutic agents include yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (^{211}At), rhenium (^{186}Re), bismuth (^{212}Bi or ^{213}Bi), and rhodium (^{188}Rh). Radioisotopes useful as labels, e.g., for use in diagnostics, include iodine (^{131}I or ^{125}I), indium (^{111}In), technetium (^{99}mTc), phosphorus (^{32}P), carbon (^{14}C), and tritium (^3H), or one or more of the therapeutic isotopes listed above.

[00198] The invention provides radiolabeled anti-PSMA antibodies and methods of labeling the same. In one embodiment, a method of labeling an anti-PSMA antibody is disclosed. The method includes contacting an anti-PSMA antibody, e.g. an anti-PSMA antibody described herein, with a chelating agent, e.g., 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA), to thereby produced a conjugated antibody. The conjugated antibody is radiolabeled with a radioisotope, e.g., $^{111}\text{Indium}$, $^{90}\text{Yttrium}$ and $^{177}\text{Lutetium}$, to thereby produce a labeled anti-PSMA antibody. Detailed procedures for radiolabeling an anti-PSMA antibody are described in more detail in the sections below and the appended examples. For example, the anti-PSMA antibodies can be radiolabeled with $^{111}\text{Indium}$, $^{90}\text{Yttrium}$, or $^{177}\text{Lutetium}$ by coupling with 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA) as described in USSN 60/295,214, filed on June 1, 2001, the contents of which are incorporated by reference in its entirety. Detailed experimental protocols for chelating anti-PSMA antibodies are described in Example 16 of USSN 60/295,214, which is specifically incorporated by reference in the present application and is reproduced below as Example 1.

[00199] As is discussed above the antibody can be conjugated to a therapeutic agent. Therapeutically active radioisotopes have already been mentioned. Examples of other

therapeutic agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, CC-1065, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol and maytansinoids).

[00200] The conjugates of the invention can be used for modifying a given biological response. The therapeutic agent is not to be construed as limited to classical chemical therapeutic agents. For example, the therapeutic agent may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, diphtheria toxin, or a component thereof (*e.g.*, a component of pseudomonas exotoxin is PE38); a protein such as tumor necrosis factor, interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Similarly, the therapeutic agent can be a viral particle, *e.g.*, a recombinant viral particle, that is conjugated (*e.g.*, via a chemical linker) or fused (*e.g.*, via a viral coat protein) to an anti-PSMA antibody of the invention. Introduction of the viral nucleic acid molecules, *e.g.*, recombinant viral nucleic acid molecules, into cells, *e.g.*, prostate cancer cells or vascular endothelial cells associated with tumors, that express PSMA can occur following binding and endocytosis of the anti-PSMA antibody/viral particle conjugate or fusion.

Nucleic Acids, Vectors and Host Cells

[00201] Another aspect of the invention pertains to isolated nucleic acid, vector and host cell compositions that can be used for recombinant expression of the modified antibodies and antigen-binding fragment of the invention. In one embodiment, a first and second isolated nucleic acid comprising a nucleotide sequence encoding heavy and light chain variable regions, respectively, of an anti-PSMA antibody, e.g., a modified anti-PSMA antibody (e.g., a deimmunized J591 or J415 anti-PSMA antibody), or an antigen fragment thereof, are provided.

[00202] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J591 immunoglobulin light chain variable region is shown in Figures 4B (SEQ ID NO:25 and 22, respectively). The non-coding complementary nucleotide sequence is also shown in Figure 4B (SEQ ID NO:26). The J591 deimmunized anti-PSMA antibody light chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-23 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:13), which is encoded by about nucleotides 261-329 of SEQ ID NO:25; a CDR1 domain corresponding to about amino acid residues 24-34 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:4), which is encoded by about nucleotides 330-362 of SEQ ID NO:25; an FR2 domain corresponding to about amino acid residues 35-49 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:14), which is encoded by about nucleotides 363-407 of SEQ ID NO:25; a CDR2 domain corresponding to about amino acid residues 50-56 of SEQ ID NO:22 (linear numbering; see SEQ ID NO:5), which is encoded by about nucleotides 408-428 of SEQ ID NO:25; an FR3 domain corresponding to about amino acid residues 57-88 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:15), which is encoded by about nucleotides 429-524 of SEQ ID NO:25; a CDR3 domain corresponding to about amino acid residues 89-97 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:6), which is encoded by about nucleotides 525-551 of SEQ ID NO:25; and an FR4 domain corresponding to about amino acid residues 98-107 of SEQ ID NO:22 (linear numbering; see also SEQ ID NO:16), which is encoded by about nucleotides 552-581 of SEQ ID NO:25.

[00203] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J591 immunoglobulin heavy chain variable region is shown in Figure 4A (SEQ ID NO:23 and 21, respectively). The non-coding complementary sequence is also shown in Figure 4A (SEQ ID NO:24). The J591 deimmunized anti-PSMA antibody heavy chain variable region

contains the following regions: an FR1 domain corresponding to about amino acid residues 1-25 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:9), which is encoded by about nucleotides 261-335 of SEQ ID NO:23; a CDR1 domain corresponding to about amino acid residues 26-35 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:1), which is encoded by about nucleotides 336-365 of SEQ ID NO:23; an FR2 domain corresponding to about amino acid residues 36-49 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:10), which is encoded by about nucleotides 366-407 of SEQ ID NO:23; a CDR2 domain of corresponding to about amino acid residues 50-66 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:2), which is encoded by about nucleotides 408-458 of SEQ ID NO:23; an FR3 domain corresponding to about amino acid residues 67-98 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:11), which is encoded by about nucleotides 459-554 of SEQ ID NO:23; a CDR3 domain corresponding to about amino acid residues 99-104 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:3), which is encoded by about nucleotides 555-572 of SEQ ID NO:23; and an FR4 domain corresponding to about amino acid residues 105-115 of SEQ ID NO:21 (linear numbering; see also SEQ ID NO:9), which is encoded by about nucleotides 573-605 of SEQ ID NO:23.

[00204] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J415 immunoglobulin light chain variable region (J415DIVK1) is shown in Figure 8A (SEQ ID NO:56 and 57, respectively). The non-coding complementary nucleotide sequence of J415DIVK1 is also shown in Figure 8A (SEQ ID NO:58). The J415 deimmunized anti-PSMA antibody light chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-23 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:41), which is encoded by about nucleotides 261-329 of SEQ ID NO:56; a CDR1 domain corresponding to about amino acid residues 24-34 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:32), which is encoded by about nucleotides 330-362 of SEQ ID NO:56; an FR2 domain corresponding to about amino acid residues 35-49 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:42), which is encoded by about nucleotides 363-407 of SEQ ID NO:56; a CDR2 domain corresponding to about amino acid residues 50-56 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:33), which is encoded by about nucleotides 408-428 of SEQ ID NO:56; an FR3 domain corresponding to about amino acid residues 57-88 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:43), which is encoded by about nucleotides 429-

524 of SEQ ID NO:56; a CDR3 domain corresponding to about amino acid residues 89-97 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:34), which is encoded by about nucleotides 525-551 of SEQ ID NO:56; and an FR4 domain corresponding to about amino acid residues 98-107 of SEQ ID NO:57 (linear numbering; see also SEQ ID NO:44), which is encoded by about nucleotides 552-581 of SEQ ID NO:56. The nucleotide and amino acid sequences of the preferred modified (deimmunized) anti-PSMA J415 immunoglobulin light chain variable region (J415DIVK5) are shown in SEQ ID NO:50 and 52, respectively; J415DIVK5 can be broken down into its component sequences in a manner identical to that shown above for J415DIVK1.

[00205] The nucleotide and amino acid sequence of the modified (deimmunized) anti-PSMA J415 immunoglobulin heavy chain variable region is shown in Figure 7A (SEQ ID NO:53 and 54, respectively). The non-coding complementary sequence is also shown in Figure 7A (SEQ ID NO:55). The J415 deimmunized anti-PSMA antibody heavy chain variable region contains the following regions: an FR1 domain corresponding to about amino acid residues 1-25 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:37), which is encoded by about nucleotides 261-335 of SEQ ID NO:53; a CDR1 domain corresponding to about amino acid residues 26-35 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:29), which is encoded by about nucleotides 336-365 of SEQ ID NO:53; an FR2 domain corresponding to about amino acid residues 36-49 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:38), which is encoded by about nucleotides 366-407 of SEQ ID NO:53; a CDR2 domain corresponding to about amino acid residues 50-68 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:30), which is encoded by about nucleotides 408-464 of SEQ ID NO:53; an FR3 domain corresponding to about amino acid residues 69-100 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:39), which is encoded by about nucleotides 465-560 of SEQ ID NO:53; a CDR3 domain corresponding to about amino acid residues 101-105 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:31), which is encoded by about nucleotides 561-575 of SEQ ID NO:53; and an FR4 domain corresponding to about amino acid residues 106-116 of SEQ ID NO:54 (linear numbering; see also SEQ ID NO:40), which is encoded by about nucleotides 576-608 of SEQ ID NO:53. The nucleotide and amino acid sequences of the preferred modified (deimmunized) anti-PSMA J415 immunoglobulin heavy chain variable region (J415DIVH4) are

shown in SEQ ID NO:51 and 49, respectively; J415DIVH4 can be broken down into its component sequences in a manner identical to that shown above for J415DIVH1.

[00206] It will be appreciated by the skilled artisan that nucleotide sequences encoding anti-PSMA modified antibodies (e.g., FR domains, e.g., FR1-4), can be derived from the nucleotide and amino acid sequences described in the present application using the genetic code and standard molecular biology techniques.

[00207] In one embodiment, the isolated nucleic acid comprises an anti-PSMA modified antibody heavy chain variable region nucleotide sequence having a nucleotide sequence as shown in Figure 4A (SEQ ID NO:23), Figure 7A (SEQ ID NO:53) or SEQ ID NO:51 (for J415DIVH4) or a complement thereof (e.g., SEQ ID NO:24 or SEQ ID NO:55), the nucleotide sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174 or a complement thereof, a sequence at least 85%, 90%, 95%, 99% or more identity thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence shown in Figure 4A (SEQ ID NO:23), Figure 7A (SEQ ID NO:53), SEQ ID NO:51, or a complement thereof (e.g., SEQ ID NO:24 or SEQ ID NO:55), or the nucleotide sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174, or a complement thereof.

[00208] In another embodiment, the isolated nucleic acid encodes an anti-PSMA modified antibody heavy chain variable region amino acid sequence having an amino acid sequence as shown in Figure 2A (SEQ ID NO:21) or Figure 5 (e.g., SEQ ID NO:49), or the amino acid sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identical thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence encoding the amino acid sequence as shown in Figure 2A (SEQ ID NO:21), Figure 5 (e.g., SEQ ID NO:49), or the amino acid sequence of the heavy chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[00209] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, and most preferably three, CDRs of the heavy chain variable region of the anti-PSMA antibody chosen from the amino acid sequences of SEQ ID

NO:1, 2, and 3, or 29, 30 and 31, or 93, 94, and 95, or 99, 100 and 101, or a CDR sequence which differs by one or two amino acids from the sequences described herein. In yet another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding CDRs 1, 2, or 3 shown in Figure 4A (SEQ ID NO:23), in SEQ ID NO:51, in Figure 7B (SEQ ID NO:125), in Figure 9A (SEQ ID NO:73), or in Figure 11A (SEQ ID NO:83), or a complement thereof, or a sequence encoding a CDR that differs by one or two amino acids from the sequences described herein.

[00210] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, three and most preferably four amino acid sequences from the heavy chain variable framework region of the anti-PSMA modified antibody chosen from SEQ ID NO:9, 10, 11 and 12, or 37, 38, 39 and 40, or a sequence at least 85%, 90%, 95%, 99% or more identical thereto.

[00211] In yet another embodiment, the isolated nucleic acid comprises an anti-PSMA modified antibody light chain variable region nucleotide sequence having a sequence as shown in Figure 4B (SEQ ID NO:25), Figure 8A (SEQ ID NO:56), or SEQ ID NO:52, or a complement thereof (e.g., SEQ ID NO:26 or 58), or the nucleotide sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identical thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to the nucleotide sequence as shown in Figure 4B (SEQ ID NO:25), Figure 8A (SEQ ID NO:56), SEQ ID NO:52, or a complement thereof (e.g., SEQ ID NO:26 or 58), or the nucleotide sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174, or a complement thereof. In another embodiment, the isolated nucleic acid encodes an anti-PSMA modified antibody light chain variable region amino acid sequence having a sequence as shown in Figure 2B (SEQ ID NO:22) or in Figure 6 (e.g., SEQ ID NO:50), the amino acid sequence of the light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; a sequence at least 85%, 90%, 95%, 99% or more identity thereto; or a sequence capable of hybridizing under stringent conditions described herein (e.g., highly stringent conditions) to a nucleotide sequence encoding the amino acid sequence as shown in Figure 2B (SEQ ID NO:22) or in Figure 6 (SEQ ID NO:50), or the amino acid sequence of the

light chain variable region of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

[00212] In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, and most preferably three, CDRs of the light chain variable region of the anti-PSMA antibody chosen from the amino acid sequences of SEQ ID NO:4, 5, and 6, or 32, 33, and 34, or 96, 97, and 98, or 102, 103, and 104, or a sequence encoding a CDR which differs by one or two amino acids from the sequences described herein.

[00213] In yet another embodiment, the isolated nucleic acid comprises a nucleotide sequence selected encoding CDRs 1-3 of the light chain variable nucleotide sequence shown in SEQ ID NO:25, or a sequence encoding a CDR which differs by one or two amino acids from the sequences described herein. In another embodiment, the isolated nucleic acid comprises a nucleotide sequence encoding at least one, preferably two, three and most preferably four amino acid sequences from the light chain variable framework region of the anti-PSMA modified antibody chosen from SEQ ID NO:13, 14, 15, and 16, or 41, 42, 43, and 44, or a sequence at least 85%, 90%, 95%, 99% or more identical thereto.

[00214] In a preferred embodiment, there is an isolated first and second nucleic acid which have nucleotide sequences encoding a light chain and the heavy chain variable regions of an anti-PSMA antibody, respectively, wherein each isolated nucleic acid has at least one, two, three, four, five and preferably all CDRs chosen from the amino acid sequences of SEQ ID NO:1, 2, 3, 4, 5, and 6, or 29, 30, 31, 32, 33 and 34, or 93, 94, 95, 96, 97, and 98, or 99, 100, 101, 102, 103, and 104, or sequence encoding a CDR which differs by one or two amino acids from the sequences described herein.

[00215] The nucleic acid can encode only the light chain or the heavy chain variable region, or can also encode an antibody light or heavy chain constant region, operatively linked to the corresponding variable region. In one embodiment, the light chain variable region is linked to a constant region chosen from a kappa or a lambda constant region. Preferably, the light chain constant region is from a lambda type (e.g., a human type lambda). In another embodiment, the heavy chain variable region is linked to a heavy chain constant region of an antibody isotype selected from the group consisting of IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA1, IgA2, IgD, and IgE. Preferably, the heavy chain constant region is from an IgG (e.g., an IgG1) isotype, e.g., a human IgG1.

[00216] Nucleic acids of the invention can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, NS0, or CHO cells.

[00217] In a preferred embodiment, the nucleic acid differs (e.g., differs by substitution, insertion, or deletion) from that of the sequences provided, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. The differences are, preferably, differences or changes at nucleotides encoding a non-essential residue(s) or a conservative substitution(s).

[00218] In one embodiment, the first and second nucleic acids are linked, e.g., contained in the same vector. In other embodiments, the first and second nucleic acids are unlinked, e.g., contained in different vectors.

[00219] In another aspect, the invention features host cells and vectors (e.g., recombinant expression vectors) containing the nucleic acids, e.g., the first and second nucleic acids, of the invention.

[00220] Prokaryotic or eukaryotic host cells may be used. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell, but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic, e.g., bacterial cells such as *E. coli*, or eukaryotic, e.g., insect cells, yeast, or preferably mammalian cells (e.g., cultured cell or a cell line). Other suitable host cells are known to those skilled in the art.

[00221] Preferred mammalian host cells for expressing the anti-PSMA antibodies, or antigen-binding fragments thereof, include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R.J. Kaufman and P.A. Sharp (1982)

Mol. Biol. 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, and a cell from a transgenic animal, e.g., e.g., mammary epithelial cell.

[00222] In another aspect, the invention features a vector, e.g., a recombinant expression vector. The recombinant expression vectors of the invention can be designed for expression of the modified antibodies, or an antigen-binding fragment thereof, in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, (1990) *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

[00223] Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to an antibody encoded therein, usually to the constant region of the recombinant antibody.

[00224] In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that are operatively linked and control the expression of the antibody chain genes in a host cell.

[00225] In an exemplary system for recombinant expression of a modified antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology

techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

Pharmaceutical Compositions

[00226] In another aspect, the present invention provides compositions, e.g., pharmaceutically acceptable compositions, which include a modified antibody molecule described herein, formulated together with a pharmaceutically acceptable carrier.

[00227] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, spinal or epidermal administration (e.g., by injection or infusion).

[00228] The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

[00229] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[00230] Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high antibody concentration. Sterile injectable solutions can be prepared by incorporating the active compound

(i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00231] The modified antibodies and antibody-fragments of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. As described in the Examples below, the anti-PSMA antibody can be administered by intravenous infusion at a rate of less than 10 mg/min, preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m², preferably about 5 to 50 mg/m², about 7 to 25 mg/m², and more preferably, about 10 mg/m². As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[00232] In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral

therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

[00233] Therapeutic compositions can be administered with medical devices known in the art.

[00234] Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00235] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. As described in Examples 10 and 12, the anti-PSMA antibody can be administered by intravenous infusion at a rate of less than 10 mg/min, preferably less than or equal to 5 mg/min to reach a dose of about 1 to 100 mg/m², preferably about 5 to 50 mg/m², about 7 to 25 mg/m², and more preferably, about 10 mg/m². It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[00236] The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the modified antibody or antibody fragment may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the modified antibody or antibody fragment is outweighed by the therapeutically beneficial effects. A "therapeutically effective dosage" preferably inhibits a measurable parameter, e.g., tumor growth rate by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit a measurable parameter, e.g., cancer, can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit, such inhibition *in vitro* by assays known to the skilled practitioner.

[00237] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[00238] Also within the scope of the invention are kits comprising an anti-PSMA antibody, preferably a modified antibody, or antigen-binding fragment thereof. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, a therapeutic agent, or an agent useful for chelating, or otherwise coupling, an antibody to a label or therapeutic agent, or a radioprotective composition; devices or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject. Instructions for use can include instructions for diagnostic applications of the anti-PSMA antibodies (or antigen-binding fragment thereof) to detect PSMA, *in vitro*, e.g., in a sample, e.g., a biopsy or cells from a patient having a cancer or prostatic disorder, or *in vivo*. The instructions can include instructions for therapeutic application including suggested dosages and/or modes of administration, e.g., in a patient with a

cancer or prostatic disorder. Other instructions can include instructions on coupling of the antibody to a chelator, a label or a therapeutic agent, or for purification of a conjugated antibody, e.g., from unreacted conjugation components. As discussed above, the kit can include a label, e.g., any of the labels described herein. As discussed above, the kit can include a therapeutic agent, e.g., a therapeutic agent described herein. The kit can include a reagent useful for chelating or otherwise coupling a label or therapeutic agent to the antibody, e.g., a reagent discussed herein. For example, a macrocyclic chelating agent, preferably 1,4,7,10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA), can be included. The DOTA can be supplied as a separate component or the DOTA (or other chelator or conjugating agent) can be supplied already coupled to the antibody. Additional coupling agents, e.g., an agent such as N-hydroxysuccinimide (NHS), can be supplied for coupling the chelator, e.g., DOTA, to the antibody. In some applications the antibody will be reacted with other components, e.g., a chelator or a label or therapeutic agent, e.g., a radioisotope, e.g., yttrium or lutetium. In such cases the kit can include one or more of a reaction vessel to carry out the reaction or a separation device, e.g., a chromatographic column, for use in separating the finished product from starting materials or reaction intermediates.

[00239] The kit can further contain at least one additional reagent, such as a diagnostic or therapeutic agent, e.g., a diagnostic or therapeutic agent as described herein, and/or one or more additional anti-PSMA antibodies (or fragments thereof), formulated as appropriate, in one or more separate pharmaceutical preparations.

[00240] The kit can further contain a radioprotectant. The radiolytic nature of isotopes, e.g., ^{90}Y (yttrium) is known. In order to overcome this radiolysis, radioprotectants may be included, e.g., in the reaction buffer, as long as such radioprotectants are benign, meaning that they do not inhibit or otherwise adversely affect the labeling reaction, e.g., of an isotope, such as ^{90}Y , to the antibody.

[00241] The formulation buffer of the present invention may include a radioprotectant such as human serum albumin (HSA) or ascorbate, which minimize radiolysis due to yttrium or other strong radionuclides. Other radioprotectants are known in the art and can also be used in the formulation buffer of the present invention, i.e., free radical scavengers (phenol, sulfites, glutathione, cysteine, gentisic acid, nicotinic acid, ascorbyl palmitate, $\text{HOP}(\cdot\text{O})\text{H}_2\text{I}$ glycerol, sodium formaldehyde sulfoxylate, $\text{Na}_2\text{S}_2\text{O}_4$, $\text{Na}_2\text{S}_2\text{O}_3$, and SO_2 , etc.).

[00242] A preferred kit is one useful for radiolabeling a chelator- conjugated protein or peptide with a therapeutic radioisotope for administration to a patient. The kit includes (i) a vial containing chelator-conjugated antibody, (ii) a vial containing formulation buffer for stabilizing and administering the radiolabeled antibody to a patient, and (iii) instructions for performing the radiolabeling procedure. The kit provides for exposing a chelator-conjugated antibody to the radioisotope or a salt thereof for a sufficient amount of time under amiable conditions, e.g., as recommended in the instructions. A radiolabeled antibody having sufficient purity, specific activity and binding specificity is produced. The radiolabeled antibody may be diluted to an appropriate concentration, e.g., in formulation buffer, and administered directly to the patient with or without further purification. The chelator- conjugated antibody may be supplied in lyophilized form.

Uses of the Invention

[00243] The modified antibodies have *in vitro* and *in vivo* diagnostic, therapeutic and prophylactic utilities. For example, these antibodies can be administered to cells in culture, e.g. *in vitro* or *ex vivo*, or in a subject, e.g., *in vivo*, to treat, prevent, and/or diagnose a variety of disorders, such as cancers (prostatic and non-prostatic cancers), as well as non-cancerous prostatic conditions (e.g., benign hyperplastic prostatic disorders).

[00244] As used herein, the term "subject" is intended to include human and non-human animals. Preferred human animals include a human patient having a disorder characterized by abnormal functioning of a PSMA-expressing cell, e.g., a cancer cell or a prostatic cell. The term "non-human animals" of the invention includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc.

[00245] In one embodiment, the subject is a human subject. Alternatively, the subject can be a mammal expressing a PSMA-like antigen with which a modified antibody of the invention cross-reacts. A modified antibody molecule of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, a modified anti-PSMA antibody (or fragment thereof) can be administered to a non-human mammal expressing the PSMA-like antigen with which the modified antibody cross-reacts (e.g., a primate, pig or mouse) for veterinary purposes or as an animal model of human disease. Regarding the latter, such

animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

Therapeutic Uses

[00246] In one embodiment, the invention provides a method of treating, e.g., ablating or killing, a cell, e.g., a prostatic cell (e.g., a cancerous or non-cancerous prostatic cell, e.g., a normal, benign or hyperplastic prostatic epithelial cell), or a malignant, non-prostatic cell, e.g., a cell found in a non-prostatic solid tumor, a soft tissue tumor, or a metastatic lesion (e.g., a cell found in renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), pancreatic (e.g., pancreatic duct) cancer and/or metastasis, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). Methods of the invention include the steps of contacting the cell, or a nearby cell, e.g., a vascular endothelial cell proximate to the cell, with a modified anti-PSMA antibody, e.g., a modified anti-PSMA antibody as described herein, in an amount sufficient to treat, e.g., ablate or kill, the cell.

[00247] The subject method can be used on cells in culture, e.g. *in vitro* or *ex vivo*. For example, prostatic cells (e.g., malignant or normal, benign or hyperplastic prostate epithelial cells) or non-prostatic cancerous or metastatic cells (e.g., renal, an urothelial, colon, rectal, lung, breast or liver, cancerous or metastatic cells) can be cultured *in vitro* in culture medium and the contacting step can be effected by adding the modified anti-PSMA antibody or fragment thereof, to the culture medium. The method can be performed on cells (e.g., prostatic cells, or non-prostatic cancerous or metastatic cells) present in a subject, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol. For *in vivo* embodiments, the contacting step is effected in a subject and includes administering the modified anti-PSMA antibody or fragment thereof to the subject under conditions effective to permit both binding of the antibody or fragment to the cell, or the vascular endothelial cell proximate to the cell, and the treating, e.g., the killing or ablating of the cell.

[00248] Examples of prostatic disorders that can be treated or prevented include, but are not limited to, genitourinary inflammation (e.g., inflammation of smooth muscle cells) as in prostatitis; benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or

hyperplasia); and cancer, e.g., adenocarcinoma or carcinoma, of the prostate and/or testicular tumors.

[00249] As used herein, the term "cancer" is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness.

[00250] Examples of non-prostatic cancerous disorders include, but are not limited to, solid tumors, soft tissue tumors, and metastatic lesions. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary tract (e.g., renal, urothelial cells), pharynx. Adenocarcinomas include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention.

[00251] The subject method can be useful in treating malignancies of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal (e.g., colon), bladder, genitourinary tract (e.g., prostate), pharynx, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

[00252] In other embodiments, the antibodies of the invention can be used for the diagnosis and treatment of a subject experiencing pain or suffering from a pain-associated disorder. Preferably, the subject is a human, e.g., a patient with pain or a pain-associated disorder disclosed herein. For example, the subject could have a disease of the prostate, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The pain can be associated with bones, as well as with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA

antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. In addition, by reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[00253] Methods of administering modified antibody molecules are described above. Suitable dosages of the molecules used will depend on the age and weight of the subject and the particular drug used. The modified antibody molecules can be used as competitive agents for ligand binding to inhibit, reduce an undesirable interaction.

[00254] In one embodiment, the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies, or antigen-binding fragments thereof, can be used to kill or ablate cancerous cells and normal, benign hyperplastic, and cancerous prostate epithelial cells *in vivo*. For example, the anti-PSMA antibodies can be used to treat or prevent a disorder described herein. The antibodies, e.g., the modified antibodies, (or fragments thereof) can be used by themselves or conjugated to a second agent, e.g., a cytotoxic drug, radioisotope, or a protein, e.g., a protein toxin or a viral protein. This method includes: administering the modified antibody, alone or conjugated to a cytotoxic drug, to a subject requiring such treatment.

[00255] Since the anti-PSMA antibodies (or fragments thereof) recognize normal, benign hyperplastic, and cancerous prostate epithelial cells, any such cells to which the modified antibodies bind are destroyed. Although such administration may destroy normal prostate epithelial cells, this is not problematic, because the prostate is not required for life or survival. Although the prostate may indirectly contribute to fertility, this is not likely to be a practical consideration in patients receiving the treatment of the present invention. In the case of cancerous tissues, since the modified antibodies recognize vascular endothelial cells that are proximate to cancerous cells, binding of the modified antibody/cytotoxic drug complex to these vascular endothelial cells destroys them, thereby cutting off the blood flow to the proximate cancerous cells and, thus, killing or ablating these cancerous cells. Alternatively, the modified antibodies, by virtue of their binding to vascular endothelial cells that are proximate to cancerous cells, are localized proximate to the cancerous cells. Thus, by use of suitable modified antibodies (including those containing substances effective to kill cells nondiscriminatingly but only over a short range), cells in cancerous tissue (including cancerous cells) can be selectively killed or ablated.

[00256] The antibodies of the present invention may be used to deliver a variety of therapeutic agents, e.g., a cytotoxic moiety, e.g., a therapeutic drug, a radioisotope, molecules of plant, fungal, or bacterial origin, or biological proteins (e.g., protein toxins) or particles (e.g., a recombinant viral particles, e.g., via a viral coat protein), or mixtures thereof. The therapeutic agent can be an intracellularly active drug or other agent, such as short-range radiation emitters, including, for example, short-range, high-energy α -emitters, as described herein. In some preferred embodiments, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a molecule of plant or bacterial origin (or derivative thereof), e.g., a maytansinoid (e.g., maytansinol or the DM1 maytansinoid, see Figure 15). DM1 is a sulfhydryl-containing derivative of maytansine that can be linked to antibodies via a disulfide linker that releases DM1 when inside target cells. The disulfide linkers display greater stability in storage and in serum than other linkers. Maytansine is a cytotoxic agent that effects cell killing by preventing the formation of microtubules and depolymerization of extant microtubules. It is 100- to 1000-fold more cytotoxic than anticancer agents such as doxorubicin, methotrexate, and vinca alkylid, which are currently in clinical use. Alternatively, the anti-PSMA antibody, or antigen binding fragment thereof, can be coupled to a taxane, a calicheamicin, a proteosome inhibitor, or a topoisomerase inhibitor. [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-[(3-mercaptoacetyl)amino]propyl]amino]butyl] Boronic acid is a suitable proteosome inhibitor. N,N'-bis[2-(9-methylphenazine-1-carboxamido)ethyl]-1,2-ethanediamine is a suitable topoisomerase inhibitor.

[00257] Enzymatically active toxins and fragments thereof are exemplified by diphtheria toxin A fragment, nonbinding active fragments of diphtheria toxin, exotoxin A (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, α -sacrin, certain *Aleurites fordii* proteins, certain Dianthin proteins, *Phytolacca americana* proteins (PAP, PAPII and PAP-S), *Morodica charantia* inhibitor, curcin, crotin, *Saponaria officinalis* inhibitor, gelonin, mitogillin, restrictocin, phenomycin, and enomycin. In a preferred embodiment, the anti-PSMA antibody is conjugated to maytansinoids, e.g., maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545). Procedures for preparing enzymatically active polypeptides of the immunotoxins are described in W084/03508 and W085/03508, which are hereby incorporated by reference, and in the appended Examples below. Examples of cytotoxic moieties that can be conjugated to the antibodies include adriamycin, chlorambucil, daunomycin, methotrexate, neocarzinostatin, and platinum.

[00258] To kill or ablate normal, benign hyperplastic, and cancerous prostate epithelial cells, a first antibody, e.g., a modified antibody, can be conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second antibody, e.g., a second modified antibody according to the present invention, preferably one that binds to a non-competing site on the prostate specific membrane antigen molecule. Whether two modified antibodies bind to competing or non-competing binding sites can be determined by conventional competitive binding assays. For example, monoclonal antibodies J591, J533, and E99 bind to competing binding sites on the prostate specific membrane antigen molecule. Monoclonal antibody J415, on the other hand, binds to a binding site that is non-competing with the site to which J591, J533, and E99 bind. Thus, for example, the first modified antibody can be one of J591, J533, and E99, and the second modified antibody can be J415. Alternatively, the first modified antibody can be J415, and the second modified antibody can be one of J591, J533, and E99. Drug-prodrug pairs suitable for use in the practice of the present invention are described in Blakely et al., "ZD2767, an Improved System for Antibody-directed Enzyme Prodrug Therapy That Results in Tumor Regressions in Colorectal Tumor Xenografts," (1996) *Cancer Research*, 56:3287-3292, which is hereby incorporated by reference.

[00259] Alternatively, the antibody, e.g., the modified antibody, can be coupled to high energy radiation emitters, for example, a radioisotope, such as ^{131}I , a γ -emitter, which, when localized at the tumor site, results in a killing of several cell diameters. See, e.g., S.E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al. (eds.), pp 303-316 (Academic Press 1985), which is hereby incorporated by reference. Other suitable radioisotopes include α -emitters, such as ^{212}Bi , ^{213}Bi , and ^{211}At , and β -emitters, such as ^{186}Re and ^{90}Y . Radiotherapy is expected to be particularly effective, because prostate epithelial cells and vascular endothelial cells within cancers are relatively radiosensitive. Moreover, Lu^{117} may also be used as both an imaging and cytotoxic agent.

[00260] Radioimmunotherapy (RIT) using antibodies labeled with ^{131}I , ^{90}Y , and ^{177}Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radionuclide can be important in order to deliver maximum radiation dose to the tumor. The higher beta energy particles of ^{90}Y

may be good for bulky tumors, but it may not be necessary for small tumors and especially bone metastases, (e.g. those common to prostate cancer). The relatively low energy beta particles of ^{131}I are ideal, but *in vivo* dehalogenation of radioiodinated molecules is a major disadvantage for internalizing antibody. In contrast, ^{177}Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to ^{90}Y . In addition, due to longer physical half-life (compared to ^{90}Y), the tumor residence times are higher. As a result, higher activities (more mCi amounts) of ^{177}Lu labeled agents can be administered with comparatively less radiation dose to marrow. There have been several clinical studies investigating the use of ^{177}Lu labeled antibodies in the treatment of various cancers. (Mulligan T et al. (1995) *Clin Cancer Res.* 1: 1447-1454; Meredith RF, et al. (1996) *J Nucl Med* 37:1491-1496; Alvarez RD, et al. (1997) *Gynecologic Oncology* 65: 94-101).

[00261] The antibodies of the invention can also be conjugated or fused to viral surface proteins present on viral particles. For example, a single-chain anti-PSMA antibody of the present invention could be fused (e.g., to form a fusion protein) to a viral surface protein. Alternatively, a whole anti-PSMA antibody of the present invention, or a fragment thereof, could be chemically conjugated (e.g., via a chemical linker) to a viral surface protein. Preferably, the virus is one that fuses with endocytic membranes, e.g., an influenza virus, such that the virus is internalized along with the anti-PSMA antibody and thereby infects PSMA-expressing cells. The virus can be genetically engineered as a cellular toxin. For example, the virus could express or induce the expression of genes that are toxic to cells, e.g., cell death promoting genes. Preferably, such viruses would be incapable of viral replication.

[00262] The antibodies, e.g., the modified antibodies of the invention, can be used directly *in vivo* to eliminate antigen-expressing cells via natural complement or antibody-dependent cellular cytotoxicity (ADCC). Modified antibody molecules of the invention, which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement can also be used in the presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with modified antibodies or fragments thereof of the invention can be improved by binding of complement proteins. In another embodiment

target cells coated with the modified antibodies or fragments thereof can also be lysed by complement.

[00263] The antibodies, e.g., the modified antibodies, of the present invention can be used and sold together with equipment, as a kit, to detect the particular label.

[00264] Also encompassed by the present invention is a method of killing or ablating cells which involves using the antibodies described herein, e.g., the modified antibodies for preventing a PSMA-related disorder. For example, these materials can be used to prevent or delay development or progression of prostate or other cancers.

[00265] Use of the therapeutic methods of the present invention to treat prostate and other cancers has a number of benefits. Since the modified antibodies according to the present invention only target cancerous cells (such as cells of cancerous tissues containing vascular endothelial cells) and prostate epithelial cells, other tissue is spared. As a result, treatment with such modified antibodies is safer, particularly for elderly patients. Treatment according to the present invention is expected to be particularly effective, because it directs high levels of modified antibodies, such as antibodies or binding portions thereof, probes, or ligands, to the bone marrow and lymph nodes where prostate cancer metastases and metastases of many other cancers predominate. Moreover, the methods of the present invention are particularly well-suited for treating prostate cancer, because tumor sites for prostate cancer tend to be small in size and, therefore, easily destroyed by cytotoxic agents. Treatment in accordance with the present invention can be effectively monitored with clinical parameters, such as, in the case of prostate cancer, serum prostate specific antigen and/or pathological features of a patient's cancer, including stage, Gleason score, extracapsular, seminal, vesicle or perineural invasion, positive margins, involved lymph nodes, disease related pain, e.g., bone pain, etc. Alternatively, these parameters can be used to indicate when such treatment should be employed.

[00266] The invention also features methods of treating pain, e.g., reducing pain, experienced by a subject having or diagnosed with prostate disease, e.g., benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, e.g., a cancer having vasculature which expresses PSMA (e.g., renal, urothelial (e.g., bladder), testicular, colon, rectal, lung (e.g., non-small cell lung carcinoma), breast, liver, neural (e.g., neuroendocrine), glial (e.g., glioblastoma), or pancreatic (e.g., pancreatic duct) cancer, melanoma (e.g., malignant melanoma), or soft tissue sarcoma). The methods include administering an anti-PSMA antibody as described herein, e.g.,

a modified anti-PSMA antibody, to a subject in an amount sufficient to treat, e.g., reduce, the pain associated with prostate disease or non-prostate cancer. The subject may have no signs of prostate disease or non-prostate cancer other than, e.g., elevated levels of serum PSA and the sensation of pain. Patients that have prostate cancer often experience bone pain, as well as, pain associated with obstructive voiding symptoms due to enlarged prostate, e.g., urinary hesitancy or diminished urinary stream, frequency or nocturia. The treatment of pain using the modified anti-PSMA antibodies of the invention can lead to a decreased or dramatically lowered need, or even eliminate the need, for analgesics, e.g., narcotics. By reducing pain, the methods of treatment can restore the mobility of, e.g., limbs, that have become dysfunctional as a result of pain associated with movement.

[00267] Because the antibodies, e.g., the modified antibodies, of the present invention bind to living prostate cells, therapeutic methods for treating prostate cancer using these modified antibodies are not dependent on the presence of lysed prostate cells. For the same reasons, diagnostic and imaging methods which determine the location of living normal, benign hyperplastic, or cancerous prostate epithelial cells (as well as vascular endothelial cells within cancers) are much improved by employing the modified antibodies of the present invention. In addition, the ability to differentiate between living and dead prostate cells can be advantageous, especially to monitor the effectiveness of a particular treatment regimen.

[00268] The antibodies, e.g., the modified antibodies, or antigen-binding portions thereof, of the present invention bind to extracellular domains of prostate specific membrane antigens or portions thereof in normal, benign hyperplastic, and cancerous prostate epithelial cells as well as vascular endothelial cells proximate to cancerous cells. As a result, when practicing the methods of the present invention to kill, ablate, or detect normal, benign hyperplastic, and cancerous prostate epithelial cells as well as vascular endothelial cells proximate to cancerous cells, the antibodies, e.g., the modified antibodies, bind to all such cells, not only to cells which are fixed or cells whose intracellular antigenic domains are otherwise exposed to the extracellular environment. Consequently, binding of the antibodies, e.g., the modified antibodies, is concentrated in areas where there are prostate epithelial cells, irrespective of whether these cells are fixed or unfixed, viable or necrotic. Additionally or alternatively, these antibodies, e.g., these modified antibodies, or binding portions thereof, bind to and are internalized with prostate

[00271] Anti-PSMA antibodies of the invention can be administered in combination with one or more of the existing modalities for treating prostate cancers, including, but not limited to: surgery (e.g., radical prostatectomy); radiation therapy (e.g., external-beam therapy which involves three dimensional, conformal radiation therapy where the field of radiation is designed to conform to the volume of tissue treated; interstitial-radiation therapy where seeds of radioactive compounds are implanted using ultrasound guidance; and a combination of external-beam therapy and interstitial-radiation therapy); hormonal therapy, which can be administered before or following radical prostatectomy or radiation (e.g., treatments which reduce serum testosterone concentrations, or inhibit testosterone activity, e.g., administering a leuteinizing hormone-releasing hormone (LHRH) analog or agonist (e.g., Lupron, Zoladex, leuprolide, buserelin, or goserelin) or antagonists (e.g., Abarelix). Non-steroidal anti-androgens, e.g., flutamide, bicalutimide, or nilutamide, can also be used in hormonal therapy, as well as steroidal anti-androgens (e.g., cyproterone acetate or megestrol acetate), estrogens (e.g., diethylstilbestrol), PROSCAR™, secondary or tertiary hormonal manipulations (e.g., involving corticosteroids (e.g., hydrocortisone, prednisone, or dexamethasone), ketoconazole, and/or aminoglutethimide), inhibitors of 5 α -reductase (e.g., finasteride), herbal preparations (e.g., PC-SPES), hypophysectomy, and adrenalectomy. Furthermore, hormonal therapy can be performed intermittently or using combinations of any of the above treatments, e.g., combined use of leuprolide and flutamide.

[00272] In other embodiments, the anti-PSMA antibodies, e.g., the modified anti-PSMA antibodies, are administered in combination with an immunomodulatory agent, e.g., IL-1, 24, 6, or 12, or interferon alpha or gamma. As described in Example 14 below, the combination of antibodies having a human constant regions and IL-2 potentially is expected to enhance the efficacy of the monoclonal antibody. IL-2 will function to augment the reticuloendothelial system to recognize antigen-antibody complexes by its effects on NK cells and macrophages. Thus, by stimulating NK cells to release IFN, GM-CSF, and TNF, these cytokines will increase the cell surface density of Fc receptors, as well as the phagocytic capacities of these cells. Therefore, the effector arm of both the humoral and cellular arms will be artificially enhanced. The net effect will be to improve the efficiency of monoclonal antibody therapy, so that a maximal response may be obtained. A small number of clinical trials have combined IL-2 with a monoclonal antibody (Albertini et al. (1997) *Clin Cancer Res* 3:1277-1288; Frost et al. (1997)

Cancer 80:317-333; Kossman et al. (1999) *Clin Cancer Res* 5:2748-2755). IL-2 can be administered by either bolus or continuous infusion. Accordingly, the antibodies of the invention can be administered in combination with IL-2 to maximize their therapeutic potential.

Diagnostic Uses

[00273] In one aspect, the present invention provides a diagnostic method for detecting the presence of a PSMA protein *in vitro* (e.g., in a biological sample, such as a tissue biopsy, e.g., from a cancerous or prostatic tissue) or *in vivo* (e.g., *in vivo* imaging in a subject). The method includes: (i) contacting the sample with a modified anti-PSMA antibody or fragment thereof, or administering to the subject, the modified anti-PSMA antibody; (optionally) (ii) contacting a reference sample, e.g., a control sample (e.g., a control biological sample, such as plasma, tissue, biopsy) or a control subject)); and (iii) detecting formation of a complex between the anti-PSMA antibody, and the sample or subject, or the control sample or subject, wherein a change, e.g., a statistically significant change, in the formation of the complex in the sample or subject relative to the control sample or subject is indicative of the presence of PSMA in the sample.

[00274] Preferably, the anti-PSMA antibody (or fragment thereof) is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials, as described above and described in more detail below.

[00275] Complex formation between the anti-PSMA antibody and PSMA can be detected by measuring or visualizing either the antibody (or antibody fragment) bound to the PSMA antigen or unbound antibody (or antibody fragment). Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. Alternative to labeling the anti-PSMA antibody, the presence of PSMA can be assayed in a sample by a competition immunoassay utilizing standards labeled with a detectable substance and an unlabeled anti-PSMA antibody. In this assay, the biological sample, the labeled standards and the PSMA binding agent are combined and the amount of labeled standard bound to the unlabeled antibody is determined. The amount of PSMA in the sample is inversely proportional to the amount of labeled standard bound to the PSMA binding agent.

[00276] In still another embodiment, the invention provides a method for detecting the presence of PSMA-expressing cancerous tissues (particularly the vascular endothelial cells therein) and normal, benign hyperplastic, and cancerous prostate epithelial cells *in vivo*. The method includes (i) administering to a subject (e.g., a patient having a cancer or prostatic disorder) an anti-PSMA antibody, preferably a modified antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the PSMA-expressing tissues or cells. Particularly preferred antibodies include modified antibodies having CDRs from any of a J591, J415, J533 or E99, and in particular deimmunized versions of these antibodies, particularly deJ591 or deJ415.

[00277] Examples of labels useful for diagnostic imaging in accordance with the present invention are radiolabels such as ^{131}I , ^{111}In , ^{123}I , $^{99\text{m}}\text{Tc}$, ^{32}P , ^{125}I , ^3H , ^{14}C , and ^{188}Rh , fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes, such as a transrectal probe, can also be employed. These isotopes and transrectal detector probes, when used in combination, are especially useful in detecting prostatic fossa recurrences and pelvic nodal disease. The modified antibody can be labeled with such reagents using techniques known in the art. For example, see Wensel and Meares (1983) *Radioimmunoimaging and Radioimmunotherapy*, Elsevier, New York, which is hereby incorporated by reference, for techniques relating to the radiolabeling of antibodies. See also, D. Colcher et al. (1986) *Meth. Enzymol.* 121: 802-816, which is hereby incorporated by reference.

[00278] In the case of a radiolabeled modified antibody, the modified antibody is administered to the patient, is localized to the tumor bearing the antigen with which the modified antibody reacts, and is detected or "imaged" *in vivo* using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. See e.g., A.R. Bradwell et al., "Developments in Antibody Imaging", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al., (eds.), pp 65-85 (Academic Press 1985), which is hereby incorporated by reference. Alternatively, a positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C , ^{18}F , ^{15}O , and ^{13}N).

[00279] Fluorophore and chromophore labeled modified antibodies can be prepared from standard moieties known in the art. Since antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluorescent compounds and chromophores are described by Stryer (1968) *Science*, 162:526 and Brand, L. et al. (1972) *Annual Review of Biochemistry*, 41:843-868, which are hereby incorporated by reference. The modified antibodies can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747, and 4,376,110, which are hereby incorporated by reference.

[00280] One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins derived from 3,6-dihydroxy-9-henylxanthhydrol and resamines and rhodamines derived from 3,6-diamino-9-phenylxanthhydrol and lissanime rhodamine B. The rhodamine and fluorescein derivatives of 9-o-carboxyphenylxanthhydrol have a 9-o-carboxyphenyl group. Fluorescein compounds having reactive coupling groups such as amino and isothiocyanate groups such as fluorescein isothiocyanate and fluorescamine are readily available. Another group of fluorescent compounds are the naphthylamines, having an amino group in the α or β position.

[00281] In cases where it is important to distinguish between regions containing live and dead prostate epithelial cells or to distinguish between live and dead prostate epithelial cells, the antibodies of the present invention (or other modified antibodies of the present invention), labeled as described above, can be coadministered along with an antibody or other modified antibody which recognizes only living or only dead prostate epithelial cells labeled with a label which can be distinguished from the label used to label the subject antibody. By monitoring the concentration of the two labels at various locations or times, spatial and temporal concentration variations of living and dead normal, benign hyperplastic, and cancerous prostate epithelial cells can be ascertained. In particular, this method can be carried out using the labeled antibodies of the present invention, which recognize both living and dead epithelial prostate cells, and labeled 7E11 antibodies (Horoszewicz et al. (1987) *Anticancer Research* 7:927-936), which recognize only dead epithelial prostate cells.

[00282] In other embodiments, the invention provide methods for determining the dose, e.g., radiation dose, that different tissues are exposed to when a subject, e.g., a human subject, is

administered an anti-PSMA antibody that is conjugated to a radioactive isotope. The method includes: (i) administering an anti-PSMA antibody as described herein, e.g., a modified anti-PSMA antibody, that is labeled with a radioactive isotope to a subject; (ii) measuring the amount of radioactive isotope located in different tissues, e.g., prostate, liver, kidney, or blood, at various time points until some or all of the radioactive isotope has been eliminated from the body of the subject; and (iii) calculating the total dose of radiation received by each tissue analyzed. The measurements can be taken at scheduled time points, e.g., day 1, 2, 3, 5, 7, and 12, following administration (at day 0) of the radioactively labeled anti-PSMA antibody to the subject. The concentration of radioisotope present in a given tissue, integrated over time, and multiplied by the specific activity of the radioisotope can be used to calculate the dose that a given tissue receives. Pharmacological information generated using anti-PSMA antibodies labeled with one radioactive isotope, e.g., a gamma-emitter, e.g., ^{111}In , can be used to calculate the expected dose that the same tissue would receive from a different radioactive isotope which cannot be easily measured, e.g., a beta-emitter, e.g., ^{90}Y .

Pharmacogenomics

[00283] With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23:983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43:254-266. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype," or "drug response genotype.") Thus, another aspect of the

invention provides methods for tailoring an individual's prophylactic or therapeutic treatment according to that individual's drug response genotype.

[00284] Information generated from pharmacogenomic research can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when administering a therapeutic composition, e.g., a composition consisting of one or more anti-PSMA antibodies, or derivatized form(s) thereof, to a patient, as a means of treating a disorder, e.g., a cancer or prostatic disorder as described herein.

[00285] In one embodiment, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies when determining whether to administer a pharmaceutical composition, e.g., a composition consisting of one or more anti-PSMA antibodies, derivatized form(s) thereof, and optionally a second agent, to a subject. In another embodiment, a physician or clinician may consider applying such knowledge when determining the dosage, e.g., amount per treatment or frequency of treatments, of a pharmaceutical composition, e.g., a pharmaceutical composition as described herein, administered to a patient.

[00286] In yet another embodiment, a physician or clinician may determine the genotypes, at one or more genetic loci, of a group of subjects participating in a clinical trial, wherein the subjects display a disorder, e.g., a cancer or prostatic disorder as described herein, and the clinical trial is designed to test the efficacy of a pharmaceutical composition, e.g., a composition consisting of one or more anti-PSMA antibodies, and optionally a second agent, and wherein the physician or clinician attempts to correlate the genotypes of the subjects with their response to the pharmaceutical composition.

Deposits

[00287] Hybridomas E99, J415, J533, and J591 have been deposited pursuant to, and in satisfaction of, the requirements of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure with the American Type Culture Collection ("A.T.C.C.") at 10801 University Boulevard, Manassas, VA 20110-2209. Hybridoma E99 was deposited on May 2, 1996, and received A.T.C.C. Designation Number HB-12101. Hybridoma J415 was deposited on May 30, 1996, and received A.T.C.C.

Designation Number HB-12109. Hybridomas J533 and J591 were deposited on June 6, 1996, and received A.T.C.C. Designation Numbers HB-12127 and HB-12126, respectively.

[00288] An NS0 cell line producing deimmunized J591 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on September 18, 2001 and assigned Accession Number PTA-3709. An NS0 cell line producing deimmunized J415 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on March 21, 2002 and assigned Accession Number PTA-4174. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

[00289] The following invention is further illustrated by the following examples, which should not be construed as further limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

EXAMPLES

Example 1 - Chelation Of Anti-PSMA Antibodies To ¹¹¹Indium, ⁹⁰Yttrium, and ¹⁷⁷Lutetium

[00290] The modified anti-PSMA monoclonal antibodies can be radiolabeled with ¹¹¹Indium, ⁹⁰Yttrium, or ¹⁷⁷Lutetium by directly coupling one of the four carboxylic acid groups of the chelator 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) to primary amines present on the surface of the antibodies. The DOTA conjugated antibody is then purified, sterile filtered, and vialled. Prior to use, the purified antibodies can be mixed with the desired radiolabel which binds to DOTA.

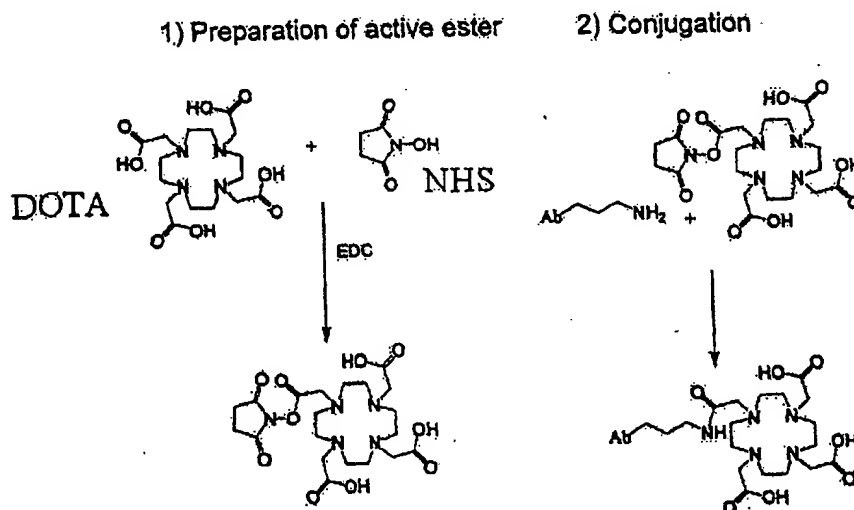
Chelation Process

[00291] Monoclonal antibody deJ591 was conjugated with 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) and subsequently radiolabeled with ¹¹¹In, ⁹⁰Y and ¹⁷⁷Lu. Radiolabeling and quality control tests were performed on three separate vials of clinical grade mAb deJ591.

[00292] All reagents used in the conjugation and purification of deJ591 were made from pyrogen-free water. In the specific case of NH_4OAc buffer and sodium phosphate buffer, the solutions were purified with Chelex 100 (Bio-Rad, CA) to remove any metal ions.

Conjugation of Antibody with 1,4,7,10-Tetraazacyclododecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetraacetic acid (DOTA)

[00293] The monoclonal antibody deJ591 was modified with 1,4,7,10-tetraazacyclododecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetraacetic acid (DOTA) as follows. Briefly, 25 mg of deJ591 was concentrated in a 30 kDa microsep centrifugal concentrator (Pall Filtron, MA) and washed with 5×4 mL of 1% DTPA (pH 5.0), over a period of 24 hours. The antibody buffer was then changed to 0.1 M phosphate (pH 7.0) using the same centrifugal technique. An active ester of DOTA was created by dissolving 146 mg DOTA (0.361 mmoles) and 36 mg N-hydroxysuccinimide (0.313 mmoles) in 2 ml of water and adjusting the pH to 7.3 with NaOH, prior to the addition of 10 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (see below).



This reaction mixture was cooled on ice for 1 hour before being added to the deJ591 solution. The resultant DOTA-deJ591 was separated from the excess DOTA and other reactants by repeated washing with 0.3 M NH_4OAc (20×4 mL) and centrifugal concentration. The purified conjugate was then sterilized by filtration through a $0.22 \mu\text{m}$ filter and stored in a sterile polypropylene vial at 4°C .

[00294] The concentration of the DOTA-deJ591 conjugate was assayed by determining the UV absorption at 280 nm and two 50 μ L aliquots mixed with either 20 or 30 μ L of a 1.30 mM solution of InCl_3 (0.01 M HCl) spiked with a tracer amount of ^{111}In . The mixture is incubated at 37°C for 16 hours and then analyzed by ITLC, using silica gel impregnated glass fiber 10 cm strip (ITLC-SG, Gelman, prod. # 61885) and an eluant of 1% DTPA (pH 6.0). The antibody bound activity remains at the origin and free ^{111}In moves with the solvent front as an ^{111}In -DTPA complex. The relative amounts of ^{111}In and ^{111}In -DOTA-J591 is determined by cutting the ITLC strip at a R_f of 0.5 and counting the two halves with a Na(Tl)I detector. The number of binding sites is calculated by considering the molar reaction ratio between ^{111}In and DOTA-deJ591 and the observed ratio of ^{111}In and ^{111}In -DOTA-J591 detected. Typically, 5.1 molecules of DOTA are conjugated to deJ591. Table 10 shows the results from two conjugations of deJ591.

Table 10: Calculation of the Mean Number of DOTA Molecules Conjugated to deJ591

Test number	Known ^{111}In /DOTA-J591	Observed ^{111}In /DOTA-J591	Mean number of DOTA mols per mAb
	Reaction ratio	TLC ratio	
A	11.76	1.338	5.03
B	17.64	2.469	5.09

Radiolabeling

[00295] The following radiolabeling procedure is described for ^{111}In , but may be used with other radiolabels such as ^{90}Y or ^{177}Lu . Radiolabeling was achieved by adding the ^{111}In (in dilute HCl) to the ammonium acetate buffered DOTA-deJ591. To avoid the effects of autoradiolysis on the antibody, the reaction time was minimized and the reaction mixture purified with a size exclusion column prior to administration. Briefly, a mixture composed of 20 μ L of $^{111}\text{InCl}_3$ (8 mCi, 0.01 M HCl, 400 μ L DOTA-deJ591 (4 mg/ml, 0.3 M NH_4OAc , pH 7) was allowed to react at 37°C for 20 minutes. The reaction mixture was then separated on a 16 mL Biogel-P6DG column (Bio-Rad, CA) equilibrated with 4 x 10 mL of sterile 1% HSA in PBS (HSA meets specification for US licensed albumin; manufactured by Central Laboratory Blood Transfusion Service Swiss Red Cross, Bern, Switzerland, License No. 647). Once the reaction mixture was loaded onto the column, it was washed with a further 2 mL of 1% HSA PBS, before

the main ^{111}In -DOTA-deJ591 fraction was eluted with 5 mL of 1% HSA PBS. The purified ^{111}In -DOTA-deJ591 was then sterile filtered into a sterile evacuated vial. Using this method, specific activity of 7.6 mCi ^{111}In /mg DOTA-deJ591 was achieved.

Alternative Radiolabeling Procedure for ^{111}In

[00296] The following radiolabeling procedure can be used for the routine preparation of ^{111}In -DOTA-J591 for clinical studies and stability studies. Radiolabeling is achieved by the addition of ^{111}In chloride and Ammonium acetate buffer (1 M) to DOTA-J591 solution (8 mg/ml, 0.3 M Ammonium acetate, pH 7). To avoid the effects of autoradiolysis on the antibody, the reaction time has been minimized. The labeled ^{111}In -DOTA-J591 is purified using a size exclusion column and sterile filtered using a 0.2m millipore membrane filter prior to administration to patients.

[00297] Briefly, ammonium acetate, (10 μL for each mCi of ^{111}In) is added to a reaction vial containing ^{111}In -chloride solution. Subsequently, the DOTA-J591 solution (30 mL or 0.24 mg for each mCi of ^{111}In) is added to the reaction vial and the mixture is gently mixed and incubated at 37°C for 20-30 min. An aliquot of the mixture is tested to determine labeling efficiency using ITLC (SG and 5 mM DTPA, pH 5). If the binding is optimal (>70%), the reaction is stopped by the addition of 10-40 mL of 5 mM DTPA.

[00298] In order to separate or purify ^{111}In -DOTA-J591 from free ^{111}In , the reaction mixture is applied on a Biogel-P6DG column (Bio-Rad, CA), prewashed with 4 x 10 ml of PBS containing 1% Human Serum Albumin (meets specification for US licensed albumin; manufactured by Central Laboratory Blood Transfusion Service Swiss Red Cross, Bern, Switzerland, License No. 647). The ^{111}In -DOTA-J591 is eluted from the column using PBS with 1% HSA and the fractions containing the labeled antibody (typically 5-8 ml) are collected into a sterile container. Following determination of radiochemical purity using ITLC (as before), and if the labeling efficiency is >95%, the labeled complex is filtered into a sterile vial using 0.2m Filter. The final specific activity is typically 3-5 mCi/mg of antibody.

Radiolabeling with ^{90}Y

[00299] The procedure is identical to the procedure described above for ^{111}In , except the incubation time is 10-15 min. Radiochemical purity of ^{90}Y -DOTA-J591 must be >97%.

Radiolabeling with ^{177}Lu

[00300] The procedure is similar to the procedure described above except for two changes. The amount of Ammonium acetate added is reduced (3-5 mL for each mCi of ^{177}Lu) and the incubation time is only 5 min. Radiochemical purity of ^{177}Lu -DOTA-J591 should be >97%.

Radiochemical Purity

[00301] The amount of free ^{111}In in radiolabeled DOTA-deJ591 preparations was evaluated using the instant thin layer chromatography method with a silica gel impregnated glass fiber support and a mobile phase of 1% DTPA (pH 5.5). Briefly, a portion of the radiolabeled DOTA-deJ591 was spotted on a 10 cm ITLC-SG strip (Gelman, prod. # 61885) and developed in 1% DTPA (pH 5.5). Once the solvent front had reached the end of the strip, it was removed from the solvent and cut at a R_f of 0.5. The two portions were assayed for radioactivity and the radiochemical purity determined using the following equation:

$$\text{Radiochemical purity} = (\text{Activity in between } R_f 0 \text{ and } 0.5) / (\text{Total activity in strip})$$

Immunoreactivity

[00302] The immunoreactivity of the ^{111}In -DOTA-deJ591 preparations was assessed according to the method of Lindmo (Lindmo T. et al. (1994) *J. Immunol. Methods*, 72:77-89, 1994) that extrapolates the binding of the radiolabeled antibody at an infinite excess of antigen. Briefly, five test solutions were prepared (in duplicate) containing 10,000 cpm of ^{111}In -DOTA-deJ591 and various amounts of LNCaP cells, in a total test volume of 250 μL of 0.2 % BSA 10 mM HEPES. The solutions were incubated at 4°C for 60 minutes prior to being isolated (by centrifugation) and washed with ice cold PBS. The membranes were then counted in a gamma counter with standards representing the total radioactivity added. The data was plotted using the Lindmo method as the reciprocal of the substrate concentration (x-axis) against the reciprocal of the fraction bound (y-axis). The data was then fitted according to a least squares linear regression method (Sigma Plot) and the y intercept taken as the reciprocal of the immunoreactivity. A similar method using membranes derived from LNCaP cells, and subsequent centrifugation

isolation of the membranes, gave similar results. The results gave an average immunoreactivity of 72% (see Table 11).

Immunohistochemistry

[00303] Immunohistochemistry was performed on the DOTA conjugated, partially purified, bulk intermediate deJ591. The results showed that the preparation was specific to prostate tissue and the reactivity was equivalent to the naked deJ591 antibody.

Sterility

[00304] The sterility of ^{111}In -DOTA-deJ591 preparations was determined using thioglycollate medium according to the method of USP 24/NF 19. Briefly, quadruplicate 0.1 mL samples of the ^{111}In -DOTA-deJ591 preparations were transferred to 15 mL of fluid thioglycollate medium and the mixture incubated at 35°C for 14 days. The media were visually inspected on the 4th, 7th and 14 days of any signs of growth. All three preparations showed no growth (See Table 11).

Endotoxin

[00305] The endotoxin of ^{111}In -DOTA-deJ591 preparations was determined using the Limulus amebocyte lysate assay according to the USP 24/NF 19. Briefly, a Limulus amebocyte lysate kit (Bio Whittaker lot # 7L3790, sensitivity 0.125EU/mL) was reconstituted with 0.25 mL of test sample. The quadruplicate test samples, artificially positive test samples, negative controls and positive controls were incubated at 37°C for 60 minutes. Positive results were typified by the formation of a viscous gel that was unaffected by 180° inversion. The single preparation gave a value of less than 5 EU/mL. This assay can (and will) be repeated on the patient dose immediately prior to administration.

Table 11: Analytical Results of Radiolabeled ^{111}In -DOTA-deJ591

Test	Result
Radiolabeling yield	85%
Radiochemical Purity	>99%
Immunoreactivity	72%
Endotoxin	<5 Eu/mL

Sterility	Sterile
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Lot # of deJ591: BIOV983.2-2

Large-Scale Manufacture/Process

[00306] The large-scale manufacture of the DOTA conjugated deJ591 antibody is described in the following paragraphs. The major differences from the above methodology were the use of a stirred cell, instead of a microsep centrifugal concentrator to concentrate and diafilter the antibody and the use of a Sephadex G-25 column to remove the unreacted DOTA and other reagents from the DOTA conjugated antibody. These changes were necessitated by the increase in scale. The ratios of the starting materials are given in Table 12 for a nominal 1000 milligram scale. The process may be scaled up using equivalent ratios of starting materials.

Table 12: Unit Ratios of Starting Materials

Starting Material	Unit Ratio
deJ591 antibody	X mgs
1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA)	1.25 X mgs
N-hydroxysuccinimide (NHS)	0.275 X mgs
1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)	0.3 X mgs

[00307] Aseptic practices were observed in order to minimize contamination and environmental monitoring was conducted at periodic intervals during the manufacture. All solutions, buffers and reagents used in the conjugation and purification of DOTA-deJ591 antibody were made with Water For Injection (WFI). Throughout the process, metal free components were used in the manufacture in order to avoid the chelation of any free metal residues by the DOTA moiety. In the specific case of ammonium acetate buffer and sodium phosphate buffer, the solutions were purified with Chelex 100 to remove any metal ions. Sterile, pyrogen free and metal free containers were used to mix reactants. The final bulk sterile filtration was conducted in an area that meets Class 100 specifications.

[00308] The deJ591 was prepared by buffer exchanging the antibody into metal free, 0.1 M Sodium Phosphate, pH 7.1, over a Chelex 100 (BioRad or equivalent) column. The antibody was then concentrated to approximately 10 mg/mL using a Stirred Cell Unit (Millipore or

equivalent) equipped with a 30kD cut-off membrane. The concentrated antibody was then sterile-filtered through a 0.22 μ m filter.

[00309] To conjugate one gram of antibody, the active ester of DOTA was prepared by adding 6.3 mL of 0.49 M DOTA in metal free, Sodium Phosphate Buffer, pH 7.1, to 2.7 mL of 0.87 M N-hydroxysuccinimide in metal free, Sodium Phosphate Buffer, pH 7.1. To this mixture, 0.1 N Sodium Hydroxide was added until the DOTA was completely dissolved (approximately a 1:1 ratio of 0.1 M Sodium Hydroxide to DOTA/NHS solution). The pH was between 6.9 and 7.2. The solution was cooled for not less than 30 minutes at 2-8°C. To the DOTA/NHS solution, 1.5 mL of 1.0 M of EDC in Sodium Phosphate Buffer, pH 7.1, was added and allowed to cool at 2-8°C for not less than 1 hour.

[00310] The active DOTA ester was added to 1 gram of antibody and incubated overnight (12-14 hrs) at 2-8°C. The DOTA conjugated antibody was purified over a Sephadex G-25 column (Pharmacia or equivalent) in metal free, 0.3 M Ammonium Acetate Buffer, pH 7.2. The eluate fraction containing the DOTA conjugated antibody was concentrated using a Stirred Cell equipped with a 30 kD cut-off membrane to approximately 10 mg/mL. The DOTA conjugated deJ591 Antibody was then diafiltered in 0.3 M Ammonium Acetate, pH 7.2 to remove any excess reagents and diluted to a final concentration of 8.0 mg/mL prior to sterile filtering through a 0.22 μ m filter.

[00311] DOTA conjugated deJ591 was tested for concentration, immunoreactivity, conjugation, endotoxin, and sterility. The endotoxin limit is based on the low clinical dose of the radiolabeled DOTA conjugated deJ591 antibody required, which ranges from 1 to 5 mg. Bioburden testing was performed on the bulk purified DOTA conjugated antibody instead of sterility because of the small batch sizes. Sterility (21 CFR 610) will be performed on the final vial drug product. The target for immunoreactivity and number of DOTA moles per antibody was based on previous clinical experience. DOTA conjugated antibody with immunoreactivity values of as low as 72% have been successfully used in the clinic. The number of DOTA moles per antibody is based on the results from previous clinical lots.

Protein Concentration

[00312] A sample of DOTA-deJ591 was analyzed by optical density in a spectrophotometer at a wavelength of 280 nm. The extinction coefficient used for these

calculations was A_{280} , $E_{1\text{ cm}}^{0.1\%} = 1.4$. The test sample was suitably diluted to give an absorbance reading in the working range of the assay (0.2 OD units to 1.2 OD units, linear, CV less than 2%). The acceptable limit for protein concentration is $8.0 \text{ mg/mL} \pm 0.5 \text{ mg/mL}$.

Endotoxin

[00313] Samples of DOTA-deJ591 were tested for pyrogens using a validated Limulus Amebocyte Lysate test (LAL) Gel Clot Assay (BioWhittaker or equivalent). A 0.06 EU/mL sensitivity Lysate was utilized and samples were diluted either 1:10 or 1:25 in Endotoxin free water for analysis in order to overcome the inhibition level of certain chemicals to the gel clot assay. Duplicate determinations were made for each buffer or intermediate sample during processing and the sample values needed to be equal to or less than the value obtained at the dilution level set for that buffer. A positive and negative control, as well as an inhibition control, was run with every sample. The proposed acceptable limits were not more than 5 EU per mg of DOTA-deJ591.

Bioburden

[00314] Aliquots of DOTA-deJ591 were directly inoculated in fluid thioglycollate and soybean-casein broth. The media were examined after fourteen days of incubation. As necessary, both media showed no growth after fourteen days.

Immunoreactivity

[00315] The immunoreactivity of the DOTA-deJ591 preparations was assessed according to the method of Lindmo (Lindmo T. et al. (1994) *J. Immunol. Methods* 72:77-89) which extrapolates the binding of the radiolabeled antibody at an infinite amount of excess antigen. Briefly five test solutions were prepared (in duplicate) containing 10,000 cpm of $^{111}\text{Indium}$ labeled-DOTA-deJ591 and various amounts of LNCaP cells or cell membranes, in a total test volume of 250 μL of 0.2 % BSA 10 mM HEPES. The solutions were incubated at 4°C for 60 minutes prior to being isolated (by centrifugation) and washed with ice cold PBS. The membranes were then counted in a gamma counter with standards representing the total radioactivity added. The data was plotted using the Lindmo method as the reciprocal of the substrate concentration (x-axis) against the reciprocal of the fraction bound (y-axis). The data

was then fitted according to a least squares linear regression method (Sigma Plot) and the y intercept used as the reciprocal of the immunoreactivity. The target for immunoreactivity was not less than 75%.

Number of DOTA Moles per antibody

[00316] The number of DOTA bound per antibody was determined using a saturation binding method with natural occurring isotope of Indium and ^{111}In . Multiple aliquots (minimum two) of DOTA-deJ591 were mixed with various amounts, ranging from 10 to 30 μL , of a 3.0 mM solution of InCl_3 (0.01 M HCl) spiked with a tracer amount of ^{111}In . The mixture was incubated at 37°C for 16 hours and then analyzed by ITLC, using silica gel impregnated glass fiber 10 cm strip (ITLC-SG, Gelman, or equivalent) and an eluant of 1% DTPA (pH 6.0). The antibody bound activity remains at the origin and free ^{111}In moves with the solvent front as an ^{111}In -DTPA complex. The relative amounts of ^{111}In and ^{111}In -DOTA-J591 was determined by cutting the ITLC strip at a R_f of 0.5 and counting the two halves with a Na(Tl)I detector. The number of binding sites was calculated by considering the molar reaction ratio between ^{111}In and DOTA-deJ591 and the observed ratio of ^{111}In and ^{111}In -DOTA-J591 detected. The target number of DOTA molecules per antibody was between 4 and 6.

[00317] The analytical results for a sample lot of DOTA conjugated deJ591 antibody are shown below in Table 13.

Table 13

Test	Proposed Acceptable Limits	Results
Appearance	Clear Colorless Solution	Clear Colorless Solution
Concentration	8.0 mg/mL \pm 0.5 mg/mL	8.4 mg/mL
Endotoxin	NMT 5 EU per mg	<1.2 EU/mg
Bioburden	No growth	No growth
Immunoreactivity	For Information Only (Target NLT 75%)	95%
Number of DOTA moles per Antibody	For Information Only (Target 4-6 DOTA per Antibody)	6

[00318] The DOTA conjugation numbers for a previous lot of DOTA conjugated antibody (Biov983.2-2) and current Lot 243101 are shown in Table 14. The average number of DOTA moles per antibody for Lot Biov983.2-2 was 5.06 and for Lot 243101 was 5.96. Although the

number of moles of DOTA conjugated per antibody was slightly higher for Lot 243101, the immunoreactivity was not affected as shown in Table 15. In fact, the immunoreactivity for Lot 243101 was higher than that for the comparison lot, which is beneficial. It should be noted that other small-scale clinical lots have had immunoreactivity values of greater than 90% (data not shown).

Table 14: Comparison of the Mean Number of DOTA Molecules Conjugated to deJ591 antibody

Lot number	Known ^{111}In /DOTA-deJ591	Observed ^{111}In /DOTA-deJ591	Mean number of DOTA mols per mAb
BIOV983.2-2	Reaction ratio	TLC ratio	
A	11.76	1.338	5.03
B	17.64	2.469	5.09
Ave			5.06
Lot 243101	Reaction ratio	TLC ratio	
A	10.98	0.8608	5.90
B	16.46	1.7301	6.03
C	21.95	2.8226	5.74
D	32.93	4.3498	6.15
Ave			5.96

A= 10 μL of In-natural/ ^{111}In solution, B= 15 μL of In-natural/ ^{111}In solution, C=20 μL of In-natural/ ^{111}In solution, D=30 μL of In-natural/ ^{111}In solution

Table 15: Comparison of Immunoreactivity of DOTA-deJ591

Test	Lot BIOV983.2-2	Lot 243101
Immunoreactivity	72%	95%

Alternatives

[00319] An alternative synthesis of the DOTA-J591 immunoconjugate is as follows: 956.5 mg of deJ591 was diafiltered six times. The antibody was concentrated in a 30 kDa microsep centrifugal concentrator (Pall Filtron, MA) to approximately 15 mg/mL and diluted 12.5 fold with metal free 0.1 M Sodium phosphate at pH 7.1. This procedure was performed six times. An active ester of DOTA was created by mixing 598 mg of DOTA (1.48 mmoles) in 5.95 mL 0.1 M metal free phosphate buffer and 132 mg N-hydroxysuccinimide (1.15 mmoles) in 2.7 ml of 0.1 M metal free phosphate buffer. The pH was adjusted to 6.9-7.2 with NaOH, prior to the addition of 144 mg (0.75 mmoles) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 1.45

mL 0.1 M metal free phosphate buffer. This reaction mixture was filtered through a 0.2 micron sterile filter and cooled on ice for 1 hour before being added to the deJ591 solution and incubated overnight at 2-8°C for 14-18 hours. The resultant DOTA-deJ591 was separated from the excess DOTA and other reactants by purifying it through a G-25 column equilibrated in 0.3 M metal free ammonium acetate. The purified conjugate was concentrated to 10 mg/mL in a stirred cell unit and washed with 0.3 M ammonium acetate, then sterilized by filtration through a 0.22 um filter and stored in a sterile polypropylene vial at 2-8°C.

[00320] Yet another alternative is to use a conjugation process that involves pure DOTA-NHS mono-active ester (commercially available from Macrocyclics) so as to achieve better control over the amount and purity of the DOTA-NHS mono-active ester used in the conjugation process, as well as to limit unanticipated chemical side-reactions produced when DOTA is activated in-situ. Problems associated with the use of in-situ activated DOTA (i.e., DOTA activated without any purification prior to being added to the antibody) include: fluctuation of DOTA/antibody incorporation ratio due to the variable generation of DOTA-NHS; the use of a large excess of DOTA which needs to be purified away from the conjugated antibody and can compete for binding to radioactive isotopes; unreacted EDAC can result in the cross-linking of lysine and glutamic or aspartic acid residues on proteins and consequently the formation of undersired protein aggregates; and there is the possibility that two or more active esters are formed on a significant fraction of the activated DOTA molecules which enables proteins to become crosslinked and uses additional carboxylate groups that are needed for metal coordination.

[00321] To minimize the loss of antibody binding activity resulting from its conjugation to DOTA, it is desirable to incorporate about 2-10, preferably about 5-7 DOTA molecules per antibody molecule. Using DOTA-NHS monoactivated ester, a 3-30 fold excess of DOTA-NHS relative to antibody produces this desired level of DOTA incorporation. Studies are currently being conducted using input ratios of DOTA-NHS to antibody of 7:1, 9:1, 11:1, 15:1, 20:1, and 30:1.

[00322] The protocol is as follows. Starting materials: J591 antibody, 10.5 mg/ml is sodium phosphate buffer (0.1M, pH 7.1), treated with Chelex 100 resin (1 ml resin per 10 mg of antibody); 0.3 M ammonium acetate buffer (pH 7.0), treated with Chelex 100 resin (20 ml resin per one liter buffer); DOTA-NHS.PF6 (FW 646.4), Macrocyclics, Dallas, TX. Experimental

procedures: Three polypropylene vials were separately charged with 2.0 ml of J591 antibody (10.5 mg/ml, 143 nmol) and chilled on ice over a period of 30 minutes. 3.3 mg of DOTA-NHS were dissolved in 0.356 ml of metal-free water (treated with Chelex 100 resin and pre-chilled on ice for 30 minutes) to give a concentration of 14.3 nmol per microliter. To the three antibody solutions were separately added 70, 90, and 110 microliters of this freshly prepared DOTA-NHS solution. The reaction mixtures were slowly stirred with magnetic stirring bars at room temperature over a period of 4 hours, and then diluted with 0.3 M ammonium acetate buffer (pH 7.0, Chelex 100 treated) to 15 ml in CentriCon-30 for buffer exchange. The concentrates (about 1.5 ml) were then diluted again to 15 ml and concentrated down to 1.5 ml. These concentrates were then filtered through 0.2 micron filters. The CentriCon tube and filters were washed with a small amount of ammonium acetate buffer and filtered into final products (total of 2.0 ml each).

[00323] Antibodies conjugated to DOTA using the 7:1, 9:1, and 11:1 input ratio of DOTA-NHS to antibody have been analyzed for ^{90}Y binding stability and the formation of protein aggregates. All three conjugates displayed a high percentage of stability after 2-3 days of labeling with ^{111}In or ^{90}Y , in the presence of PBS, DTPA chelate challenger, serum and transferrin. In addition, all three conjugates displayed little or no process-related aggregate formation. The remaining three conjugates, produced using the higher DOTA-NHS to antibody input ratios, are currently being analyzed.

[00324] The conjugation of DOTA to antibodies is not limited to NHS activated DOTA. The DOTA-HOBT active ester can be used in place of DOTA-NHS, as well as other activation methods known in the art, such as the use of a mixed anhydride of ethyl chloroformate or isobutyl chloroformate, p-nitrophenyl ester.

Example 2- Pharmacokinetics and Biodistribution of ^{131}I - and ^{111}In -labeled deJ591 and murine J415 in Nude Mice Bearing LNCaP Human Prostate Tumors

[00325] In nude mice bearing PSMA-positive human LNCaP tumors, the pharmacokinetics, biodistribution and tumor uptake of monoclonal deJ591 and murine J415 antibodies radiolabeled with ^{131}I or ^{111}In was analyzed. Autoradiographic studies were performed to identify intra-tumoral distribution of radiolabeled MAbs.

[00326] De J591 and J415 were labeled with ^{131}I using the iodogen method (see Franker and Speck (1978), *Biochem Biophys Res Commun* 80:849-57) to a specific activity of

400MBq/mg (21, 23). For ^{111}In labeling, the J415 and deJ591 antibodies were first conjugated with 1,4,7,10-tetraazacyclododecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetraacetic acid (DOTA) and then labeled with ^{111}In to produce specific activities of 200 MBq/mg.

[00327] Prostate carcinoma cell lines LNCaP, DU145 and PC3 (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640, supplemented with 10 % fetal calf serum, at a temperature of 37°C in an environment containing 5% CO_2 . Prior to use, the cells were trypsinized, counted and suspended in Matrigel (Collaborative Biomedical Products, Bedford, MA). Nu/Nu Balb C mice 8-10 weeks of age were inoculated, in the right and left flanks, with a suspension of 5×10^6 LNCaP cells in Matrigel (BD Biosciences, Bedford MA). After a period of 14-18 days, tumors (100-300 mg) had developed. The PSMA-negative DU145 and PC3 cells were implanted in nude mice in an identical manner.

[00328] The PSMA-positive and PSMA-negative tumor bearing mice were injected, via the tail vein, with 80 KBq of the iodinated MAb (400 MBq/mg) in 200 μL of PBS (pH 7.4, 0.2% BSA). Groups of animals (3-8/group) were sacrificed after 2, 4 or 6 days. The major organs and tumors were recovered. The tissue samples were weighed and counted, with appropriate standards in an automatic NaI(Tl) counter. These measured relative activity data (cpm) were background corrected and expressed as a percentage of the injected dose per gram (%ID/g). These data were also fitted with a least squares regression analysis (Microcal Origin, Northampton, MA) to determine the biological clearance of the various agents. J415, J591 and 7E11 were labeled with ^{111}In (100MBq/mg). 80KBq of the ^{111}In labeled MAbs were injected in groups of animals. The %ID/g in various organs and tumor tissues was determined at 2-6 days post injection in a similar way as described for ^{131}I labeled MAbs.

[00329] For the imaging studies, animals were injected with 2 MBq of ^{111}In -DOTA-J591. On days 1, 2, 3, 4 and 6 post-injection, the mice were sedated with ketamine/xylazine 100 mg/kg/10 mg/kg IP. The mice were imaged with a gamma camera (Transcam, ADAC Laboratories, Milpitas, CA) equipped with a pinhole collimator. The images were acquired in a 256×256 matrix for 1000 seconds using a 20% window of 245 KeV photopeak of ^{111}In .

[00330] For several animals ($n=20$), harvested tumor samples were immediately cooled in liquid nitrogen and frozen in embedding medium (O.C.T. 4583, Sakura Finetec, Torrance CA). Twenty micron sections were cut and the tumor sections were either fixed with acetone and placed in direct contact with a sheet of photographic film (Biomax, Kodak, Rochester, NY) or

stained with hematoxin/eosin prior to exposure of the film. Tumors from control animals were collected and cut into 10 μm sections. These sections were soaked in tris buffer (170 mM, pH 7.4, with 2 mM CaCl_2 and 5 mM KCl) for 15 minutes, washed with Tris buffer (170 mM, pH 7.4) and incubated with ^{131}I -J591 MAb for 1 hour at 4°C . Non-specific binding was determined in the presence of 100 nM J591 MAb. These sections were then washed 3 times with PBS (containing 0.2% BSA) and once with Tris buffer prior to being fixed with acetone and then exposed to photographic film.

Biodistribution of ^{131}I labeled MAbs

[00331] In nude mice bearing LNCaP tumors, the biodistribution and tumor uptake of ^{131}I labeled deJ591 and J415 was compared to that of ^{131}I -7E11. At 2 days post injection deJ591, J415, and 7E11 had similar tumor uptake and blood pool activity. On day 6, the tumor uptake of J415 (15.4 ± 1.1) was significantly higher compared to that of deJ591 (9.58 ± 1.1). The blood activity of both deJ591 and J415 was significantly lower compared to that of 7E11. With the ^{131}I labeled MAbs, the tumor/blood and tumor/muscle ratios were significantly higher with J415 than with J591 or with 7E11.

[00332] In order to assess the specificity of radiolabeled MAb localization in PSMA-positive LNCaP tumors, the uptake of ^{131}I labeled deJ591 and J415 in selected organs was compared to that of an irrelevant IgG antibody. At one day post injection, the tumor uptake (%injected dose/gram) of both J415 (12.2 ± 3.24) and deJ591 (8.55 ± 1.29) was significantly higher compared to that of an irrelevant antibody (4.41 ± 0.40). The uptake in lungs, kidney, muscle was similar with all three antibodies. In a second control study, the tumor uptake of ^{131}I -deJ591 was determined in nude mice bearing the PSMA-negative prostate tumors (PC3 and DU145). At 4 days post injection, the tumor uptake of ^{131}I -deJ591 was only 0.66 ± 0.07 % ID/g in PC3 tumors ($n=10$) and 0.55 ± 0.03 %ID/g in DU145 tumors ($n=6$). In contrast, the tumor uptake of ^{131}I -J591 was 11.4 ± 1.49 % ID/g in PSMA-positive LNCaP tumors, significantly greater than in PSMA-negative tumors ($p < 0.01$).

Biodistribution of ^{111}In labeled MAbs

[00333] With ^{111}In , the tumor uptake of deJ591 and J415 gradually increased with time and is quite similar to that of 7E11. The kinetics of blood clearance for both J415 and J591 is

faster compared to 7E11. At 6 days post-injection, the blood activity of J415 (2.63 ± 0.23) and deJ591 (2.52 ± 0.16) is about 50% that of 7E11 (4.16 ± 0.21). As a consequence, the tumor/blood ratios with J415 and deJ591 are significantly higher compared to that of 7E11. There were minor differences in the uptake of these three antibodies in liver, spleen and kidney.

[00334] The serial gamma camera images of a nude mouse clearly show the intense tumor accumulation of ^{111}In -DOTA-deJ591. On day 1, the single tumor (ca 250 mg) on the right hind quarter, the blood pool and the liver are well visualized. But in the later images, while the activity has cleared from the blood pool, the tumor accumulation become gradually more intense compared to that of liver activity.

Autoradiography

[00335] Tumor specimens ($n = 20$) were harvested for hematoxylin (H) and eosin (E) staining and autoradiography to study the intra-tumoral biodistribution of ^{131}I -labeled MAbs 4 to 6 days after intravenous injection. The H and E stains reveal a considerable amount of necrosis, averaging 50% of the cross-sectional area, in all specimens studied. The autoradiographs reveal a focal, somewhat heterogeneous, distribution pattern with all three antibodies. Interestingly, the biodistribution pattern with MAbs to PSMA_{int} and PSMA_{ext} reveal almost reciprocal patterns. That is, 7E11 (anti-PSMA_{int}) distinctly favors localization to areas of necrosis whereas J415 and J591 (anti-PSMA_{ext}) demonstrate a distinct preferential accumulation in areas of viable tumor. Ex vivo autoradiography, where ^{131}I -J591 was incubated directly on the tissue section, demonstrated a homogeneous binding pattern.

Conclusions

[00336] The localization of radiolabeled J591 and J415 in PSMA positive LNCaP tumors is highly specific. These results clearly demonstrate that PSMA-specific internalizing antibodies such as deJ591 and deJ415 may be the ideal MAbs for the development of novel therapeutic methods to target the delivery of beta emitting radionuclides (^{131}I , ^{90}Y , ^{177}Lu) for the treatment of PSMA-positive tumors.

[00337] MAbs to PSMA_{ext} had a reciprocal pattern to 7E11, with localization concentrated in areas of viable tumor. The inability of 7E11 to target well vascularized, viable tumor sites probably explains the inability of ProstaScint® to image bone metastases as well as to explain its

failure in RIT trials. By targeting viable tumors, mAbs to PSMA_{ext}, like deJ591 and deJ415, will have a better therapeutic effect. In addition, their ability to target viable tumor imparts better ability to localize well-vascularized sites in the bone marrow.

Example 3 - Animal Studies Using ⁹⁰Y-DOTA-deJ591

[00338] In *in vitro* and *in vivo* animal models, ⁹⁰Y-DOTA-deJ591 has demonstrated substantial anti-tumor activity. In these studies, immunodeficient 'nude' mice were implanted intramuscularly with PSMA-expressing human prostate cancer cells (LNCaP). In some studies, the same animals were simultaneously implanted in the opposite thigh with a PSMA-absent human prostate cancer line (PC3). Cancers were allowed to 'establish' for a period of approximately 2 weeks during which time the cancer develops a blood supply allowing further growth. At the time of treatment initiation, the cancer implants average 1.0 cm in diameter (or approximately 5% of the animal's body weight).

[00339] Four groups of mice received a single injection of 1.3, 3.7, 5.55 or 7.4 MBq of ⁹⁰Y-DOTA-deJ591. At a 1.3 MBq dose level, there was a mixed response with minimal reduction in tumor growth rate. However, at doses between 3.7-7.4 MBq, a clear anti-tumor dose-response relationship was observed. There was a 30, 55 and 90% reduction in mean tumor volume and a progressive dose-related delay in tumor re-growth of 10, 35 and 60 days at 3.7, 5.55, and 7.4 MBq dose levels, respectively. More than 70% of mice that received 3.7-5.55 MBq lived significantly longer than the controls and the mean survival time (MST) increased to 80-100 days compared to 40 days for controls.

[00340] Three groups of mice received 1.11, 2.22 or 3.33 MBq of ⁹⁰Y-DOTA-huJ591 every 28 days for 3 doses. At the 1.11 MBq dose level, there was minimal tumor growth during a period of 75-80 days. At 2.22 and 3.33 MBq dose levels, there was a 50-70% reduction in the mean tumor size at day 60 followed by gradual increase in tumor size thereafter. The MST increased by about 200% at 1.11 and 2.22 MBq dose levels compared to control group (120 vs. 40 days).

[00341] These studies confirm significant improvement in survival of the ⁹⁰Y-DOTA-deJ591 treated animals. The PSMA-absent cancers do not respond to treatment demonstrating the specificity of the treatment.

Example 4 - Animal Studies Using ^{177}Lu -DOTA-deJ591

[00342] Nude mice bearing LNCaP tumors treated with ^{177}Lu -DOTA-deJ591 exhibited a response similar to the ^{90}Y -DOTA-deJ591 treated animals. In this study mice with LNCaP tumors (300-400 mg) were divided into three groups. A control group received no treatment. Group-2 received 200 μCi and Group-3 received 300 μCi of ^{177}Lu -DOTA-deJ591. ^{177}Lu -DOTA-deJ591 was, in a dose dependent manner, able to reduce the mean tumor mass by 80-97% at the two dose levels. The control group showed a progressive increase in tumor size, which was accompanied by a steady loss of body weight and the mice were sacrificed by fifty-three days because of low body mass. The animals treated with 200 μCi ^{177}Lu -DOTA-deJ591 had tumor shrinkage up to twenty days post injection, and thereafter tumor regrowth was seen in some animals. The same group also had a nadir in body mass of about 90% (of baseline wt) at twenty days post injection, but thereafter a steady rise in the mean body weight to 100-105% of the starting mass. At ninety days, four out of eleven mice had no palpable tumors. The mice treated with 300 μCi of ^{177}Lu -DOTA-deJ591 had tumor shrinkage up to forty days post injection, and thereafter tumor regrowth was seen in some animals. The same group also had a nadir in body mass of about 90% at twenty days, but thereafter a steady rise in the mean body weight to 105-110 % of the starting mass. At ninety days, five out of eleven mice had no palpable tumors.

Example 5 - Human Trial with ^{131}I -J591 (murine): Phase I Clinical Trial Targeting A Monoclonal Antibody (mAb) To The Extracellular Domain Of Prostate Specific Membrane Antigen (Psm_{ext}) In Hormone-Independent Patients

[00343] Hormone-independent patients with rising PSA levels and acceptable hematologic, hepatic and renal function received a single dose of murine mAb J591. Doses are escalated (from 0.5-300 mg) in groups of three to six patients. Each dose included ≤ 1.0 mg J591 labeled with 10 mCi ^{131}I iodine as a tracer plus "cold" mAb. The dose levels used ranged from 0.5 to 300.0 mg as follows: 0.5 mg, 1.0 mg, 2.0 mg, 5.0 mg, 10 mg, 25 mg, 50 mg, 100 mg, 125 mg, 150 mg, 200 mg, 250 mg, and 300 mg. Blood and urine were collected to monitor pharmacokinetics, toxicity and human anti-mouse antibody (HAMA) response. Patients were imaged on the day of injection (day 0), as well as days 2, 4 and 6 to track mAb targeting.

[00344] Thirty-three patients with hormone-independent prostate cancer were entered into the phase I biodistribution trial of trace-labeled ^{131}I -J591. These patients received doses ranging

from 0.5 to 300.0 mg, in each case conjugated with 10mCi ^{131}I . In approximately 80% of patients where disease sites had been imaged by conventional studies (CT/MRI and/or bone scan), known sites of prostate cancer metastases can be imaged. Both soft tissue and bony sites could be visualized on mAb scan. Targeting was specific for prostate cancer sites without apparent localization to non-cancer sites. The mean effective serum residence time of ^{131}I -mJ591 was determined to be 44.0 hours (median 47.4 hrs). Eight out of sixteen evaluable patients developed a human anti-murine antibody (HAMA) response. That is, their immune systems recognized the foreign nature of the mouse-derived antibody. Only one patient had an adverse event related to the murine antibody. This patient had a severe allergic (anaphylactic) reaction to the murine mAb later determined to be due to prior (unknown) exposure to murine antibody used in purification of another experimental drug with which the patient had previously been treated. In sum, thirty-three patients were evaluated for targeting: twenty-seven out of thirty-three patients had positive bone scans, with twenty-four out of twenty-seven (about 90%) having positive monoclonal antibody scans; nine out of thirty-three patients had positive soft tissue (CT scans), with eight out of these nine (about 90%) having positive monoclonal antibody scans.

[00345] Development of a HAMA response, which occurs in most immunocompetent patients who receive murine antibodies, precludes repeated treatments with a foreign species-derived antibody. In order to allow for multiple treatments, murine antibody molecules can be "de-immunized" using molecular engineering techniques which remove foreign (mouse) amino acid sequences and replace them genetically with known homologous human sequences. As indicated above, murineJ591 has undergone de-immunization resulting in "deJ591".

[00346] In conclusion, mAb to PSMA_{ext} targets *in vivo* specifically to disseminated prostate cancer sites in both bone and soft tissue with no significant 'adverse' localization and no significant toxicity.

Example 6 - Single Human Patient Study Using ^{90}Y -DOTA-deJ591

[00347] A single patient was treated with mAb deJ591. This patient had bulky, poorly differentiated prostate cancer and had failed multiple courses of external beam radiotherapy as well as multiple forms of hormonal and non-hormonal chemotherapy. The patient was treated under a single patient IND and received a total of twelve doses of deJ591 over a course of five months, ranging from 10 mg to 200 mg. Four doses (#1, 3, 6 and 11) were trace-radiolabeled

with either ^{131}I or ^{111}In for pharmacokinetic determinations and biodistribution. The mean effective serum residence time of ^{131}I -deJ591 ranged from 31.9 to 51.3 hours, depending on the dose. Tumor localization to known tumor sites was excellent after each of the radiolabeled doses over the five month period. Dose twelve was radiolabeled with a therapeutic quantity of ^{90}Y (19mCi) calculated to deliver less than a 150 rad dose to the blood. This dose was determined in consultation with the FDA and took into account prior radiotherapy delivered by external beam and by ^{131}I . No detectable human anti-deimmunized antibody (immune) response developed in this patient. The patient's platelet count dropped to 64,000 (normal: 150,000-300,000) at five weeks post ^{90}Y -DOTA-deJ591 administration (as an anticipated result of radiation to the bone marrow) prior to spontaneously returning to normal levels. No other hematologic or non-hematologic toxicity occurred. The patient experienced no side effects. The patient's PSA declined from 63 at the time of deJ591-DOTA- ^{90}Y administration to 36. No measurable reduction in tumor number or size occurred. This patient succumbed to his metastatic prostate cancer ten and one-half months after initiating treatment with ^{90}Y -DOTA-deJ591.

Example 7 - Human Trial with ^{111}In -DOTA-deJ591 - Phase I Trial of ^{111}In labeled deimmunized Monoclonal Antibody (mAb) deJ591 to Prostate Specific Membrane Antigen/Extracellular Domain (PSMAext)

[00348] This example describes the results of a clinical trial of deJ591 to assess mAb targeting, toxicity, pharmacokinetics (PK) and immunogenicity (human anti-deimmunized Ab) of this genetically engineered mAb. DeJ591 is a strong mediator of antibody dependent cellular cytotoxicity (ADCC). As PSMA is expressed in tumor, but not normal, vascular endothelium of all cancers, the diagnostic and therapeutic utility of deJ591 may extend beyond prostate cancer to other cancers.

[00349] Patients with recurrent, progressing prostate cancer received four weekly doses of ^{111}In -DOTA-deJ591. Doses were escalated in cohorts of three patients and ranged from 62.5-500 mg/m² (total). Dose level is shown in Table 16. Each dose included 0.02-1.0 mg deJ591 trace-labeled with 0.1-5 mCi ^{111}In via a mAb-DOTA chelate, with the remainder of the dose consisting of unlabeled deJ591. After the first dose, patients were imaged on the day of injection (day 0) and days 1, 2, 4 and 7.

Table 16: ^{111}In -DOTA-deJ591 Dosage

Dose Level	Loading Dose (mg/m ²)	Maintenance Dose (mg/m ²)	Total Dose (mg/m ²)
1	25	12.5	62.5
2	50	25	125
3	100	50	250
4	200	100	500

1-2mg ^{111}In -DOTA-deJ591 (0.2-10 mCi), with the balance of the dose as "cold" deJ591.

[00350] Serum PK, immune reaction and toxicity were evaluated after each dose for a minimum of 12 weeks.

[00351] Fifteen patients were initially entered in the trial. Thirteen patients received all four planned doses; two patients received ≤ 1 dose. One patient became hypotensive 5 minutes into his first infusion due to a rapid infusion rate. The second patient who did not complete treatment was withdrawn from the study after one week due to rapid disease progression rendering him no longer eligible. Neither this latter patient nor the remaining thirteen patients experienced any toxicity or side effects. Ten out of the thirteen patients had positive bone scans; all ten demonstrated excellent mAb targeting to bony sites. Three patients had soft tissue disease as measured by a CT scan; two demonstrated mAb targeting to these sites, while the third patient that was ^{111}In -DOTA-deJ591 negative had a radiated pelvic mass that had not changed size in >18 months. No mAb targeting to non-prostate cancer sites was noted. None of the patients developed an immune reaction to the antibody. Plasma half-life of deJ591 varied with dose.

[00352] In conclusion, deJ591 is non-immunogenic and targets sensitively and specifically to both bone and soft tissue.

Example 8 - Human Trial with ^{111}In -DOTA-deJ591: Targeting of Hormone Refractory Prostate Cancer

[00353] This example describes the results of a clinical trial of deJ591 to assess mAb targeting, biodistribution, and pharmacokinetics and to optimize antibody dose for radioimmunotherapy with this mAb in patients exhibiting hormone refractory prostate cancer.

[00354] Twenty-six patients exhibiting hormone refractory prostate cancer were injected with a single dose of ^{111}In -DOTA-deJ591, consisting of 20mg deJ591 labeled with 185 MBq of ^{111}In -DOTA. All patients underwent whole body and SPECT imaging on days 0 (the day of injection), 3, and 6 of ^{111}In -DOTA-deJ591 injection. ^{111}In -DOTA-deJ591 imaging results were compared with CT, MRI, and bone scan. All patients had rising PSA levels on three consecutive measurements.

[00355] Twenty-two of the twenty-six patients had imagable disease on routine imaging modalities, while four patients had no imagable disease. Imaging data revealed ^{111}In -DOTA-deJ591 tumor targeting in sixteen of the twenty-two (72.7%) patients who had imagable disease. Targeting was best observed on day 3. No additional (unknown) sites were detected on SPECT imaging. Targeted metastatic sites were in the bone or bone marrow in twelve patients, in the soft tissue in two patients, and in both the bone and soft tissue in two patients.

[00356] ^{111}In -DOTA-deJ591 imaging was false negative in four of the twenty-two (18%) patients who had imagable disease. Non-targeted metastatic sites were in the soft tissue in three patients and in both the bone and soft tissue in one patient.

[00357] Accumulation of ^{111}In -DOTA-deJ591 in the prostate gland was generally minimal in patients with intact but irradiated prostate. Among physiological sites, highest uptake was observed in the liver, with $27 \pm 1.7\%$ uptake at day 6. The absorbed dose by the liver was 2.8 ± 0.25 rads/mCi with ^{111}In and 20.1 ± 2.1 rads/mCi with ^{90}Y . Plasma clearance ($T_{1/2}$) of ^{111}In -DOTA-deJ591 was 34 ± 5 hours.

[00358] ^{111}In -DOTA-deJ591 specifically targets hormone refractory prostate tumors and is an effective vehicle to target hormone refractory advanced prostate cancer with radioactivity or cytotoxins.

Example 9 - Targeting of Hormone Refractory Prostate Cancer with ^{111}In -DOTA-deJ591 or ^{177}Lu -DOTA-deJ591

[00359] Imaging studies with ^{177}Lu -DOTA-deJ591, like those described in Example 8 for ^{111}In -DOTA-deJ591, have produced similar results. The following summary represents a combination of the results obtained for ^{177}Lu and ^{111}In .

[00360] Thirty-nine patients with hormone-refractory prostate cancer have thus far been enrolled in one of several clinical trials utilizing deJ591. Nuclear imaging with either ^{111}In or

^{177}Lu labeled deJ591 was performed as an initial step of each trial. ^{111}In , which is a γ -emitter, was used either as tracer for "naked" mAb or as a surrogate imaging agent prior to the administration of ^{90}Y (a pure β emitter which does not image on radionuclide scans). ^{177}Lu emits both γ and β particles, allowing direct imaging, and can be used as both an imaging and a therapeutic agent. The antibody scans were compared to standard imaging studies (bone, CT or MR scans) obtained on each patient to assess the underlying sensitivity, specificity, and accuracy of deJ591 targeting to metastatic sites. Bony and soft tissue metastases were evaluated independently.

[00361] Results have been obtained for bony metastasis in twenty-three patients, and soft-tissue metastasis in twenty-five patients. Fifteen patients had bone metastases on bone scan, which were accurately targeted by deJ591 in twelve patients (80%). In these twelve patients, every cancerous lesion seen on bone scan was identified on the deJ591 scan. All seven patients with negative bone scans were negative on the deJ591 scan (100% specificity). For soft tissue metastases, eight out of nine patients with soft tissue masses on conventional imaging demonstrated accurate targeting using deJ591 (89%). The one false negative patient had retroperitoneal adenopathy measuring 8mm not seen on mAb scan, but whose bony lesions were all targeted. Fifteen patients without documented soft tissue metastases had negative mAb scans (100%). One patient without soft tissue metastases on initial standard imaging but lesions identified on mAb scan subsequently proved positive on standard imaging. Overall, there was a 91% concordance between standard imaging and mAb scans for soft tissue and bony metastases.

[00362] DeJ591 accurately targets known bony or soft tissue metastases in the vast majority of patients. Additionally, one previously unseen metastatic site was demonstrated on mAb scan and later confirmed with CT imaging. DeJ591 is a highly sensitive and specific agent for targeting metastatic prostate cancer lesions.

Example 10 - Human Trial with ^{90}Y -DOTA-deJ591; Phase I Trial of De-immunized mAb deJ591-DOTA- ^{90}Y trium In Patients With Relapsed Prostate Cancer

[00363] A phase I trial of escalating doses of ^{90}Y -DOTA-deJ591 therapy of patients with recurrent/relapsing prostate cancer was conducted. Doses started at 5 mCi/m² and were escalated in increments of +2.5-5 mCi/m² for cohorts of three to six patients. The design of this study is summarized as follows: ^{111}In -DOTA-deJ591 was administered to patients so as to determine the

biodistribution of the antibody and the associated dosimetry; and ^{90}Y -DOTA-deJ591 was administered 7-10 days later at 5.0, 10, 15, 17.5, and 20 mCi/m². All administrations were by intravenous infusion at a rate of about 5mg/min. DeJ591-DOTA was labeled at a specific activity between 3-5mCi- ^{90}Y /mg antibody to reach the defined dose of ^{90}Y , with the balance brought up to 20 mg total deJ591 with "cold" deJ591. Dosages were administered with 6-8 weeks between dose levels. Subsequent doses of ^{90}Y -DOTA-deJ591 were allowed.

[00364] The subjects for this trial had prostate cancer that had relapsed after definitive therapy (e.g. surgery and/or radiation) and for whom no curative standard therapy exists.

[00365] The objectives of this trial are to: (1) define the toxicity and maximum tolerated dose (MTD) of repeated (fractionated) doses of de-immunized monoclonal antibody (mAb) deJ591-DOTA- ^{90}Y trium (^{90}Y) in patients who have recurrent and/or metastatic prostate cancer; (2) define the pharmacokinetics of deJ591-DOTA- ^{90}Y ; (3) define the human anti-deimmunized antibody immune response to deJ591-DOTA- ^{90}Y ; and (4) define the preliminary efficacy (response rate) of repeated (fractionated) doses of deJ591-DOTA- ^{90}Y .

Treatment Protocol:

[00366] Patients who developed \geq grade 2 allergic reaction as a result of ^{90}Y -DOTA-deJ591 would not receive further DOTA-deJ591 and would be followed for toxicity.

[00367] Patients were followed for a minimum of 12 weeks after the deJ591-DOTA- ^{90}Y administration. If the patient's disease was stable or responding at 12 weeks after his last dose, he was followed until progression.

[00368] The follow-up study consisted of gathering the information shown in Table 17, below, at the indicated times.

Table 17: Follow-up Analyses

Medical history	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Physical exam (focused)	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Performance status	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
PSA, PAP, alkaline phosphatase	Day of Rx, week 1, 2, 4, 6, 8, and 12, then every 12 weeks thereafter until progression
Testosterone	Week 4, then every 6 months thereafter until

	progression
Human anti-deimmunized Ab	Day of Rx, week 2, 4, 8, and 12
CBC, differential, platelet count	Day of Rx, week 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, and 8, then every 4 weeks thereafter until stable, and then every 12 weeks until progression. Monitor qod if ANC <1000 and/or platelets <50,000
Electrolytes, BUN, creatinine	Day of Rx, week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Total protein, albumin, bilirubin, GGTP, AST, ALT, LDH	Day of Rx, week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Urinalysis	Day of Rx, week 1, 2, and 4, then every 4 weeks unless normal or baseline
CT or MRI	Week 12, then every 12 weeks (if measurable or evaluable disease present at entry and/or if patient classified as a "responder")
Bone scan	Week 12, then every 12 weeks (if evaluable disease present at entry and/or if patient classified as a "responder")
CXR	Week 12, then every 12 weeks (if disease present at entry)
Weight	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Appetite	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Bone pain	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Analgesic intake	Day of Rx, week 1, 2, 4, 8, and 12, then every 12 weeks until progression

Pharmacokinetics:

[00369] Following injection of ^{111}In -DOTA-deJ591, blood samples were obtained at 10 min, 1, 2, 4, 24 hours and days 2, 3, 4 and 7. The percent injected dose (% I.D.) in blood was determined by measuring an aliquot of blood along with a known ^{111}In standard. Similar blood samples were taken at the same interval after the ^{90}Y -DOTA-deJ591. The % I.D. in blood was determined by measuring an aliquot of blood along with a known ^{111}In or ^{90}Y standard.

Toxicity:

[00370] NCI CTEP Common Toxicity Criteria (CTC), version 2 (April, 1999) was utilized. Since CTEP has standardized the CTC, the NCI does not require inclusion of the CTC

within this document. All treatment areas have access to a copy of the CTC version 2.0. A copy may also be downloaded from the CTEP web site.

Definition of Dose Limiting Toxicity (DLT):

[00371] Hematologic toxicity: grade 4 granulocytopenia [ANC <500/mm³]; grade 4 thrombocytopenia [platelet count < 10,000/mm³]; or febrile neutropenia or neutropenic infection as defined by the CTC. Other toxicity: grade \geq 3 non-hematologic toxicity attributable to ⁹⁰Y-DOTA-deJ591.

Definition of Maximum Tolerated Dose (MTD):

[00372] The MTD is defined as the dose level at which 0/6 or 1/6 patients experience DLT with the next higher dose level having 2 or more patients experiencing DLT. Once the MTD has been reached, at least 6 patients should be evaluated at that dose to better determine the toxicities of the regimen and the pharmacokinetics of ⁹⁰Y-DOTA-deJ591.

[00373] Allergic events will be managed as follows: rash, pruritis, urticaria and wheezing will be treated with benadryl and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms can be treated with steroids and/or epinephrine as clinically indicated.

Specific interventions solely for the purpose of the study:

[00374] Other than the actual administration of deJ591 and related studies to define the pharmacokinetics and biodistribution of the mAb, the other interventions (labs, imaging studies, office visits) performed are standard procedures. Some of the lab tests would not typically be done in the setting of prostate cancer (e.g., immune reaction levels). Other lab and radiographic procedures, although standard in the management of patients with prostate cancer, may be done at greater frequency than typical.

Criteria for therapeutic response:

[00375] Prostate cancer progression is manifest by rising PSA levels, new lesions on bone scan, new disease-related symptoms and, less commonly, increasing size of a measurable soft

tissue mass. Response is commonly assessed either biochemically (PSA change) or by change in size of a measurable lesion/s.

[00376] Biochemical (PSA) response can be determined by comparing the nadir PSA level after therapy to the baseline, pre-treatment PSA determined just prior to initiating therapy. A decline of >50% has been demonstrated by numerous investigators (Petrylak, D.P. et al. (1992) *Cancer* 70:2870-78; Kelly W.K., et al. (1999) *J Clin Oncol* 11:607-15; Kantoff P.W., et al. (1999) *J Clin Oncol* 17:2506-13, 1999; Smith, D.C., et al. (1998) *J Clin Oncol* 16:1835-43) to correlate with improved survival. In addition, Scher, et al (Scher H.I., et al. (1999) *JNCI* 91:244-51) have demonstrated that a PSA which either declines or shows no increase from baseline at either 8 or 12 weeks after initiating therapy correlates with improved survival compared to patients whose PSA rises despite therapy.

[00377] In patients with measurable disease: complete response is defined as complete disappearance of all measurable lesions by physical examination or imaging studies with no appearance of new lesions for ≥ 2 months. Partial response: is defined as a 50% or greater reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions. There may be no new lesions. Stable disease: patients who do not meet the criteria of partial response and who are without signs of progressive disease for ≥ 2 months. Progressive disease is defined as a greater than 25% increase in the sum of the products of the longest perpendicular diameters of the indicator lesions, the appearance of new lesions or a rise in prostate specific antigen above pre-treatment baseline.

[00378] Duration of response: typically, the first sign of progression will be a rise in serum PSA. In this trial the duration of response will be the time interval from treatment initiation (^{90}Y -DOTA-deJ591) until progression is documented by either a confirmed rise in PSA, enlargement of the measurable lesion/s, or new lesion/s on imaging studies. The rising PSA must be confirmed by a second, serially rising PSA and the duration will be defined as the time from initiation of treatment to the time of the first rising PSA.

Results:

[00379] Twenty patients have been treated according to this protocol, and nineteen of the twenty can currently be evaluated. All twenty patients had failed one or more forms of hormone therapy, and eleven of the twenty patients had failed at least one chemotherapy regimen.

Furthermore, the patients all had increasing PSA levels and a minimum platelet count of 150,000.

[00380] Blood chemistry, hematology and PSA levels were monitored for twelve weeks or longer. No significant changes were observed in blood chemistry, or liver or kidney functions. Hematological changes in platelets and white blood cell levels were observed at all dose levels. Toxicity was dose-related and limited to reversible myelosuppression (primarily thrombocytopenia). Grade 3-4 thrombocytopenia was observed at 15-20 mCi/m². The maximum tolerated dose was estimated to be less than or equal to 20 mCi/m². ⁹⁰Y-deJ591 radiation dosimetry estimates based on ¹¹¹In-deJ591 imaging studies indicate that the organ dose to liver, kidney, spleen, and bone marrow are 20, 19, 18, and 1.7 rads/mCi, respectively. No patients developed an immune reaction.

[00381] Dose-related anti-tumor effects were noted. At the first two dose levels (5 and 10 mCi/m²), five out of the eleven (45%) patients had PSA values that continued to increase despite treatment, while six out of the eleven (55%) patients achieved an average 23% reduction in PSA levels. At 15 mCi/m², one out of the four patients (25%) progressed, while three out of the four (75%) patients achieved an average 25% reduction in PSA levels. At 20 mCi/m², all four patients achieved an average 42% reduction in PSA levels. Two of these four patients have PSA declines of 70-85% continuing beyond 3 months, as shown in Figures 13A and B. Mean time to PSA nadir was 7 weeks post-treatment (range: 2-13 weeks). Measurable responses have also been seen in these patients. The patient in whom the PSA level declined by 85% had 90% shrinkage of multiple soft tissue metastases. The patient with the 70% decline in PSA had a measurable decrease in soft tissue disease of 40%.

Conclusions:

[00382] ⁹⁰Y-DOTA-deJ591 is non-immunogenic (which would allow for repeated treatments) and toxicity has been limited to dose-related, reversible myelosuppression. Importantly, ⁹⁰Y-DOTA-deJ591 has dose-related anti-tumor effects in patients with advanced prostate cancer. Phase I data indicates that a single administration of ⁹⁰Y-DOTA-deJ591 (less than or equal to 20 mCi/m²) is safe with optimal dosimetry for the treatment of prostate cancer. In addition, the radiation dosimetry estimates indicate that multiple administrations are also safe.

Example 11 - Evidence of PSA Responses in Prostate Cancer Patients Receiving ^{90}Y -deJ591

[00383] Two patients receiving ^{90}Y -DOTA-deJ591 had rising PSA levels prior to treatment with radiolabeled J591 (see Figures 13A and 13B). The X-axis on the plots represents time (in days). Negative numbers on this axis indicate days prior to J591 treatment. At the "0" time point, the patients received ^{90}Y -J591 for therapy. The graphs demonstrate that the rapidly rising PSA prior to treatment takes a sharp turn within a few weeks of treatment and becomes stable for a long period of time thereafter (at least ten weeks). The stability of the PSA level indicates that the progressive disease has stopped progressing. Higher doses of radiolabeled J591 may lead to a decrease in the disease burden and/or a prolongation of the cessation of tumor growth rate. In addition, repeated doses may also lead to absolute declines in the tumor burden as well as substantial prolongation of cessation of tumor growth rate.

Example 12 - Human Trial with ^{177}Lu -DOTA-deJ591: Phase I Trial of De-immunized mAb deJ591-DOTA- ^{177}Lu In Patients With Relapsed Prostate Cancer

[00384] This example describes a clinical study of subjects who have prostate cancer that has relapsed after definitive therapy (e.g., surgery and/or radiation) and/or who are hormone independent and for whom no standard therapy exists. There is currently no curative therapy for these patients. Furthermore, the example focuses on ^{177}Lu labeled deJ591. ^{177}Lu is both a beta- and a gamma-emitter. As such, it can be used for both radiotherapy and imaging.

[00385] The objectives of this trial were to: (1) define the toxicity and maximum tolerated dose (MTD) of de-immunized monoclonal antibody (mAb) deJ591-DOTA- ^{177}Lu (^{177}Lu) in patients with prostate cancer who have recurrent and/or metastatic prostate cancer (Pca); (2) define the pharmacokinetics of deJ591-DOTA- ^{177}Lu ; (3) define the biodistribution and dosimetry of deJ591-DOTA- ^{177}Lu ; (4) define the human anti-de-immunized antibody (immune) response to deJ591-DOTA- ^{177}Lu ; and (5) define the preliminary efficacy (response rate) of deJ591-DOTA- ^{177}Lu .

Treatment Protocol:

[00386] Patients received a dose of deJ591-DOTA- ^{177}Lu administered at an infusion rate of $\leq 5\text{mg/min}$. The total dose of deJ591 remained fixed at 10 mg/m^2 . The ^{177}Lu dose (in mCi/m^2) was escalated in cohorts of three to six patients for each dose level (see Table 15

below). DeJ591-DOTA- ^{177}Lu was labeled at a specific activity between 3-10 mCi/mg antibody to reach the defined dose of ^{177}Lu , with the balance brought up to 10/m² mg total deJ591 with "cold" deJ591. Dose escalation was withheld until at least three patients at the ongoing dose level had been followed for ≥ 6 weeks without serious hematologic toxicity. If any of the initial three patients at a dose level experience grade 1 or 2 hematologic toxicity by 6 weeks, escalation was withheld until recovery began or until 8 weeks of further monitoring and evaluation of toxicity had occurred. If any patient experienced grade 3 or 4 hematologic toxicity, at least six patients needed to be entered at that dose level and followed for a minimum of 8 weeks or until recovery begins prior to escalation. If, at any time, two instances of dose-limiting toxicity were observed at a given dose level, further entry at that dose level will be halted. In such a case, at least 6 patients should be entered at the prior dose level to aid in defining MTD (see "Toxicity" section below).

[00387] Patients who develop \geq grade 2 allergic reaction while receiving ^{177}Lu -DOTA-deJ591 did not receive further deJ591 and were followed for toxicity.

Table 18

Dose Level	Total deJ591*	^{177}Lu Dose
1	10 mg/m ²	10 mCi/m ²
2	10 mg/m ²	15 mCi/m ²
3	10 mg/m ²	30 mCi/m ²
4	10 mg/m ²	45 mCi/m ²
5	10 mg/m ²	60 mCi/m ²
6	10 mg/m ²	75 mCi/m ²
7	10 mg/m ²	90 mCi/m ²
8	10 mg/m ²	105 mCi/m ²

*consisting of deJ591-DOTA- ^{177}Lu at specific activity between 3-10 mCi/mg with the balance to 10 mg/m² total with "cold" deJ591.

[00388] Patients were followed for a minimum of 12 weeks after the deJ591-DOTA- ^{177}Lu administration. If the patient's disease was stable or responding at 12 weeks after his treatment, he was followed until progression.

[00389] Except as noted in Table 16, follow-up was as described above in Table 14.

Table 16

Human anti-deimmunized Ab	Day of deJ591-DOTA- ¹⁷⁷ Lu Rx, post-Rx week 1, 2, 4, 8, and 12, then every 12 weeks until progression
Chem-7 (including Electrolytes, BUN, creatinine, glucose)	Day of deJ591-DOTA- ¹⁷⁷ Lu Rx, post-Rx week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
Liver panel (including: albumin, bilirubin (tot & dir), AST, ALT, (alkaline phosphatase)	Day of deJ591-DOTA- ¹⁷⁷ Lu Rx, post-Rx week 1, 2, 4, and 8, then every 4 weeks until stable, and then every 12 weeks until progression
LDH	Day of deJ591-DOTA- ¹⁷⁷ Lu Rx, post-Rx week 1, 2, 3, and 8, then every 4 weeks until stable, and then every 12 weeks until progression

¹⁷⁷Lu-deJ591 Imaging:

[00390] Total body images were obtained within 1 hour post-infusion (day 0) and at least 5 additional time points in the subsequent 2 weeks (e.g., days 1, 3, and 5, 10, 15). The gamma camera images were obtained using a dual head ADAC gamma camera fitted with an appropriate collimator. The percent injected dose (% I.D.) in major organs (heart, liver, spleen, kidneys, bone marrow, GI tract and bladder) was estimated by drawing regions of interest (ROI) and determining the relative counts in each organ and kinetics of wash out from each organ. SPECT studies were sometimes performed on the abdomen, pelvis and/or areas of suspected metastatic lesions. Where possible, using known standards of ¹⁷⁷Lu, percent injected dose in tumor was estimated per gram of tumor mass.

Toxicity:

[00391] NCI CTEP Common Toxicity Criteria (CTC), version 2 (April, 1999) was utilized. Since CTEP has standardized the CTC, the NCI does not require inclusion of the CTC within this document. All treatment areas have access to a copy of the CTC version 2.0. A copy may also be downloaded from the CTEP web site.

[00392] Three to six patients were entered (or will be entered) at each dose level. Dose escalation was withheld until at least three patients at the ongoing dose level had been followed for 6 weeks without hematologic toxicity. If any of the initial three patients at a dose level experience grade 1 or 2 hematologic toxicity by 6 weeks, escalation will be withheld until 8 weeks to further evaluate toxicity. If any patient experiences grade 3 or 4 hematologic toxicity,

at least six patients had to be entered at that dose level and followed for a minimum of 8 weeks prior to escalation. If, at any time, two instances of grade 3 or grade 4 toxicity were observed at a given dose level, further entry at that dose level would be terminated.

Definition of Dose Limiting Toxicity (DLT):

[00393] Hematologic toxicity: Grade 4 granulocytopenia (ANC < 500/ ul) for > 7 days or grade 4 thrombocytopenia (platelets < 10,000). Other toxicity: grade \geq 3 non-hematologic toxicity attributable to ^{177}Lu -DOTA-deJ591.

Adverse Event Definition:

[00394] An adverse event is defined as any untoward medical occurrence in a research patient during a clinical trial or 4 weeks-posttreatment, regardless of causality. This includes clinical or laboratory findings, inter-current illness or an exacerbation or progression of a disease or a condition present at the time of entry (baseline). An adverse event is non-serious if it does not meet any of the serious criteria (see below).

Causality/Attribution:

[00395] All clinical adverse events and abnormal laboratory values were evaluated by for potential relationship to the experimental agent. The following categorizes of causality/attribution will be utilized: definite, probable, possible, unlikely, and unrelated.

[00396] Abnormal clinical laboratory values of clinical significance which were present at baseline and did not change in severity or frequency during the experimental therapy and/or which can reasonably be attributed to the underlying disease were evaluated by the investigator and recorded in the "unrelated" category. Such events, therefore, were not be considered in the evaluation of the safety of this agent.

Preexisting Conditions:

[00397] In this trial, a preexisting condition (that is, a disorder present before the adverse event reporting period started) is not reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period.

Adverse Event Definitions:

[00398] Each adverse event was classified as serious or non-serious and/ expected or unexpected. An adverse event is classified as serious if it: it resulted in death; it was life-threatening (i.e., the encountered adverse event placed the subject at immediate risk of death; it does not apply to an adverse event which hypothetically might have caused death if it had been more severe); it required or prolonged in-patient hospitalization; it resulted in persistent or significant disability or incapacity; and it resulted in a congenital anomaly/birth defect.

Grading:

[00399] Toxicity was graded on a scale of 0-4 using either the Common Toxicity Criteria scales or the following:

- (1) 0 = no toxicity.
- (2) 1 = mild toxicity, usually transient, requiring no special treatment and generally not interfering with patient activity.
- (3) 2 = moderate toxicity which may be ameliorated by simple therapeutic measures; impairs usual activities.
- (4) 3 = severe toxicity requiring therapeutic intervention and interrupting usual activities. Hospitalization may or may not be required.
- (5) 4 = life threatening toxicity which requires hospitalization.
- (6) 5 = a fatal toxicity.

Criteria for therapeutic response:

[00400] Prostate cancer is manifest by rising PSA levels, new lesions on bone scan, new disease-related symptoms and, less commonly, increasing size of a measurable soft tissue mass. Response is commonly assessed either biochemically (PSA change) or by change in size of a measurable lesion/s.

[00401] Biochemical (PSA) response was determined as described above. In patients with measurable disease: Complete response is defined as complete disappearance of all measurable lesions by physical examination or imaging studies with no appearance of new lesions for > 2 months. Partial response: is defined as a 50% or greater reduction in the sum of the products of

the longest perpendicular diameters of all measurable lesions. There may be no new lesions.

Stable disease: patients who do not meet the criteria of partial response and who are without signs of progressive disease for > 2 months. Progressive disease is defined as a greater than 25% increase in the sum of the products of the longest perpendicular diameters of the immeasurable lesions, the appearance of new lesions or a rise in prostate specific antigen above pre-treatment baseline.

[00402] Duration of response: Typically, the first sign of progression will be a rise in serum PSA. In this trial the duration of response will be the time interval from treatment initiation until progression is documented by either a rise in PSA, enlargement of the measurable lesion/s, or new lesion/s on bone scan. The rising PSA must be confirmed by a second, serially rising PSA and the duration will be defined as the time from initiation of treatment to the time of the first rising PSA.

Results:

[00403] Hormone refractory patients with CT/Bone scan documented prostate cancer lesions and increasing PSA levels were enrolled in a Phase I dose-escalation (10-75 mCi/m²) study. All of the patients had failed one or more forms of hormone therapy.

[00404] To date, nineteen patients (three groups of patients, 3-6/group) have received ¹⁷⁷Lu-DOTA-deJ591 (10 mg/m²). Each group received a different dose of ¹⁷⁷Lu-DOTA-deJ591: 10, 15, 30, 45, or 60 mCi/m² (0.37, 0.74, 1.11, 1.48, or 1.85 GBq, respectively). Blood samples were obtained for two weeks, and imaging studies were performed five times during the same two weeks. Blood chemistry, hematology, and PSA levels were monitored for three months or longer.

[00405] Imaging studies showed specific tumor localization of ¹⁷⁷Lu-DOTA-deJ591. Four patients had previously unrecognized metastatic foci demonstrated upon ¹⁷⁷Lu-DOTA-deJ591 imaging, which was subsequently confirmed by conventional imaging. The radiation dosimetry estimates show that the liver is the critical organ receiving 7.6+/-3.3 rads/mCi. Dose to bone marrow based on blood activity is 0.7 rads/mCi. Plasma T1/2 of ¹⁷⁷Lu-DOTA-deJ591 was 43+/-11 hours. No significant changes were observed in blood chemistry, or liver or kidney function. Hematological changes were observed at different dose levels, but even at the 60 mCi/m² dose level, no serious toxicity was observed.

[00406] The first nine patients that entered the study received a dose of ^{177}Lu -DOTA-deJ591 at levels of 10 mCi/m² (three patients), 15 mCi/m² (three patients), and 30 mCi/m² (three patients). All patients had failed one or more forms of hormone therapy, and one (17%) patient had failed at least one chemotherapy regimen. Toxicity was minimal, with no patients having grade 2 or 3 adverse events, and limited to reversible myelosuppression, primarily thrombocytopenia. No patients developed an immune response to ^{177}Lu -DOTA-J591.

[00407] Among these first nine patients, dose-related anti-tumor effects were noted. Following treatment at the 10 mCi/m² dose level, two patients had PSA levels that continued to rise despite treatment, while the remaining patient showed stabilization of PSA levels. At 15 mCi/m², two patients had a mean decrease of 35% in PSA levels, while one patient's PSA levels progressed despite therapy. Mean time to PSA nadir was 4 weeks post treatment (range: 2-6 weeks). One of the three patients that exhibited a decrease in PSA levels, who did not have measurable disease, continued to have 50% reduced PSA levels even at 18 weeks post-treatment (see Figure 14). Some of these patients are currently being retreated and have received at least three doses of ^{177}Lu -DOTA-deJ591.

Conclusions:

[00408] ^{177}Lu -J591 is non-immunogenic (which would allow for repeated treatments) and has low toxicity at doses used thus far. It has dose-related anti-tumor effects in patients with advanced prostate cancer. In contrast to ^{90}Y -DOTA-deJ591, which has a maximum tolerated dose estimated to be about 20 mCi/m², ^{177}Lu -DOTA-deJ591 is safe even at a 30 mCi/m² dose level. ^{177}Lu -DOTA-deJ591 appears to eliminate disadvantages associated with both ^{131}I -DOTA-deJ591 (which is dehalogenated in vivo and is not ideal for mAbs that are internalized) and ^{90}Y -DOTA-deJ591. The longer residence time of ^{177}Lu -DOTA-deJ591 in tumor tissue may also augment the anti-tumor response.

Example 13 - Imaging Non-Prostate Cancers

[00409] In addition to prostate epithelial cells, immunohistochemical studies show that PSMA is also expressed by vascular endothelial cells of numerous solid tumors, but not by normal vascular endothelium in benign tissues. As discussed above, this expression pattern of PSMA occurs in virtually all solid tumors.

[00410] An IRB approved Phase I dose escalation trial of ^{111}In -DOTA-deJ591 was initiated to assess its value as an therapeutic agent for vasculotoxic therapy, to define its toxicity and maximum tolerated dose, to determine its pharmacokinetics and biodistribution, and to assess for immunogenicity. Eligible patients were those with refractory solid tumor malignancies whose tumor types are known to express PSMA on the neovasculature.

[00411] Fifteen patients received 5mg (three patients), 10mg (six patients), or 20mg (six patients) of ^{111}In -DOTA-deJ591, followed by a second dose 14 days later.

[00412] Patients the participated in the study included eight renal cell cancer patients, four bladder cancer patients, two colon cancer patients, and one pancreas cancer patient. All patients underwent whole body and SPECT imaging on days 0 (the day of injection), 2, 5, and 7 of ^{111}In -DOTA-deJ591 injection. The ^{111}In -DOTA-deJ591 imaging results were compared with CT and bone scans.

[00413] Imaging data revealed ^{111}In -DOTA-deJ591 tumor targeting in ten of the fifteen patients (7 renal cell cancer, 2 bladder cancer, and 1 colon cancer). Targeting was best observed on days 2 and 5, and was independent of antibody mass delivered. No additional sites were detected on SPECT. Targeted metastatic sites included: lungs, femur, retroperitoneal and cervical lymph nodes. Brain metastasis in one patient (with renal cell cancer) was first detected by ^{111}In -DOTA-deJ591 imaging and later confirmed by MRI. ^{111}In -DOTA-deJ591 imaging was false negative in five of the fifteen patients (2 bladder cancer, 1 colon cancer, 1 pancreas cancer, and 1 renal cell cancer). Non-targeted metastatic sites included: liver, renal bed, pancreas, lungs, and celiac lymph nodes. Undetected lung lesions measured less than 1cm in size.

[00414] No objective responses occurred, although a colon cancer patient had a 50% decline in CEA and two patients had improvement in cancer pain and performance status.

[00415] Two different patients with metastatic kidney cancer, having disease that had spread to the lungs, lymph nodes, and/or bones, were injected with deJ591 labeled with ^{111}In dium. Images of the patients, taken within 2 days of injection, demonstrated significant uptake of the antibody in the known tumor sites in these varying tissues and organs. The extent of the uptake of antibody is both substantial and rapid.

[00416] Among physiological sites, highest uptake was observed in the liver. Plasma clearance and liver uptake were dependent upon antibody mass; lower mass resulted in faster plasma clearance and higher liver uptake (in terms of percentage uptake). Plasma clearance

(1 1/2) for 5mg, 10mg, and 20mg of deJ591 were 21+/-11 hours, 24+/-6 hours, and 37+/-8 hours, respectively. Liver uptake for the same dose levels was 28%+/-14%, 17%+/-7%, and 13%+/-5%, respectively.

[00417] Based on these data, the protocol was revised to provide dosing for 6 consecutive weeks (10, 20, 40 and 80 mg/week dose levels) with the option for re-treatment on 8 week cycles if patients have stable or responding disease. To date, nine patients are currently receiving treatment on this schedule.

[00418] ¹¹¹In-DOTA-deJ591 specifically targets vascular endothelium of solid tumors. These trials demonstrate that deJ591 is an effective approach to targeting solid tumor vascular endothelium with radioactivity or cytotoxins.

Example 14 - Phase II Trial of mAb deJ591 in combination with Low-dose Subcutaneous Interleukin-2 in Patients with Recurrent Prostate Cancer

[00419] The subjects for this trial have prostate cancer which has relapsed after definitive therapy (e.g. surgery and/or radiation) and/or who are hormone independent and for whom no standard therapy exists. There is currently no curative therapy for these patients.

[00420] The objectives of the trial are to: (1) To define the preliminary efficacy (response rate) of mAb deJ591 in combination with daily low-dose subcutaneous IL-2 in patients who have recurrent and/or metastatic prostate cancer; (2) to study the toxicity of mAb deJ591 in combination with daily low-dose subcutaneous IL-2; and (3) to measure in vitro the effect of IL-2 on the immune response.

Low-Dose IL-2 Therapy:

[00421] IL-2 promotes the proliferation and enhances the secretory capacity of all major types of lymphocytes, including T cells, B cells, and natural killer (NK) cells (Smith K.A. (1988) *Science* 240:169). The IL-2 stimulated expansion of antigen-selected T-cell and B-cell clones determines the magnitude of antigen-specific immune responses, while the quality of the response is determined by IL-2 promoted secretion of additional cytokines, cytolytic molecules and antibodies (Smith K.A (1993) *Blood* 81:1414-1423). In addition, through its effects on NK cells, IL-2 stimulates antigen-nonspecific host reactions that involve an interplay between NK cells and monocytes. As a result of these functions, it follows that IL-2 should be useful as an

immune stimulant, particularly in cancer immunotherapy. The therapeutic use of IL-2, however, is made difficult because one of its major effects consists of the stimulation of secondary cytokine secretion by IL-2-responsive cells. Many of the potential beneficial effects of IL-2 can be attributed to these secondary cytokines that recruit and activate additional cell types, especially monocytes, that contribute to the total immune/inflammatory reaction. However, these same secondary cytokines, when produced in too large amounts, can also lead to severe toxicity. IL-2 was first used in very high doses for the treatment of cancer, equivalent to 150 million units (MU) of Chiron Corporation IL-2 (Rosenberg S.A (1990) *Sci. Am* 262:62-69). This high dose was determined using the dose-escalation and dose-intensification principles of chemotherapy, and was associated with significant grade 3 and 4 toxicity. IL-2 in high doses is known to cause serious side effects including fever, rigors, malaise, myalgia, nausea/vomiting, hypotension and possibly death.

[00422] In the 1990s, researchers began to examine the immunomodulatory effects and toxicities of continuous low dose IL-2 (Smith K.A (1993) *Blood* 81:1414-1423; Caligiuri et al. (1991) *J Clin Oncol* 9:2110-2119; Caligiuri et al. (1993) *J Clin Invest* 91:123-132; Bernstein et al. (1995) *Blood* 86:3287-3294). These studies demonstrated that doses of IL-2 as low as 1.2 MU daily resulted in the specific expansion of NK cells with minimal toxicity. Bernstein et al. (1995) *Blood* 86:3287-3294; Lalezari et al. (2000) HIV Clin Trials). Potential side effects include injection site reactions (usually redness at the injection site), asthenia, flu-like symptoms, nausea, diarrhea and eosinophilia. The selective expansion of human CD3⁺, CD56⁺ NK cells during low-dose IL-2 begins within 2 weeks of therapy and plateaus after 4-6 weeks of treatment (Smith K.A (1993) *Blood* 81:1414-1423; Fehniger et al. (2000) *J Clin Invest* 106:117-124). NK cells can account for as many as 60%-80% of PBMCs after one month of therapy (Smith K.A (1993) *supra*). Recent studies suggest that increased NK cell number results from enhanced NK-cell differentiation from bone marrow progenitors, combined with a delay in IL-2 dependent NK-cell death (Fehniger et al. (2000) *supra*). The low-dose IL-2 regimens have been specifically designed to completely avoid toxicity.

Combination Monoclonal Antibody and IL-2 Therapy:

[00423] The combination of monoclonal antibodies and IL-2 potentially should enhance monoclonal antibody efficacy. IL-2 will function to augment the reticuloendothelial system to

recognize antigen-antibody complexes by its effects on NK cells and macrophages. Thus, by stimulating NK cells to release IFN, GM-CSF, and TNF, these cytokines will increase the cell surface density of Fc receptors, as well as the phagocytic capacities of these cells. Therefore, the effector arm of both the humoral and cellular arms will be artificially enhanced. The net effect will be to improve the efficiency of monoclonal antibody therapy, so that a maximal response may be obtained. A small number of clinical trials have combined IL-2 with a monoclonal antibody (Albertini et al. (1997) *Clin Cancer Res* 3:1277-1288; Frost et al. (1997) *Cancer* 80:317-333; Kossman et al. (1999) *Clin Cancer Res* 5:2748-2755). In such studies, IL-2 was administered intravenously by either bolus or continuous infusion. Toxicity was associated with higher doses of IL-2.

IL-2 Therapy in Prostate Cancer:

[00424] A variety of studies have examined the effects of IL-2 on prostate cancer cells *in vitro* and in prostate cancer animal models, Moody et al. Interleukin-2 transfected prostate cancer cells generate a local antitumor effect *in vivo* (*Prostate*, 24: 244-251, 1994; Sokoloff et al. (1996) *Cancer*, 77: 1862-1872; Triest et al. (1998) *Clin Cancer Res*, 4: 2009-2014; Hautmann et al. (1999) *Anticancer Res*, 19: 2661-2663; Hillman et al. (1999) *Cancer Detect.Prev.* 23: 333-342), although there have been few clinical trials of IL-2 in patients with advanced prostate cancer. Hillman et al. (1999) *supra*; Maffezzini et al. (1996) *Prostate*, 28: 282-286; Morris et al. (2000) *Cancer*, 89: 1329-1348). A Phase II trials of I.V. intermediate dose IL-2 (dose ranging from 10-15 MU daily x 4) in patients with hormone refractory prostate cancer was conducted. In six out of ten patients, a transient decline in PSA levels was observed. It is unclear if this was from anti-tumor activity, or if IL-2 affected PSA expression, as had been reported *in vitro* studies using LNCaP cells (Sokoloff et al. (1996) *supra*). No regression of measurable disease was observed in any patient. The effect of daily sub-cutaneous low-dose IL-2 in patients with progressive prostate cancer has not been examined.

Specific Aims:

- (1) To define the preliminary efficacy (response rate) of mAb deJ591 in combination with daily low-dose subcutaneous IL-2 in patients who have recurrent and/or metastatic prostate cancer.

- (2) To study the toxicity of mAb deJ591 in combination with daily low-dose subcutaneous IL-2.
- (3) To measure the effect of IL-2 on the peripheral blood mononuclear cell (PBMC) population.
- (4) To measure in vitro the effect of IL-2 activated NK cells from patients on this protocol to induce ADCC with mAb deJ591.

Treatment Protocol:

[00425] Patients receive daily low-dose subcutaneous rIL-2 (1.2×10^6 IU/m²/day) on day 1 through day 56. After three weeks of IL-2 administration, patients receive deJ591 by I.V. (25 mg/m²) once a week for three consecutive weeks (on days 22, 29, and 36). IL-2 administration is continued during this period another two weeks afterwards. This 8-week regimen constitutes one cycle of therapy. Patients are evaluated for response at the end of one cycle. Patients who have responded to therapy or have stable disease are eligible for additional cycles of therapy. Additional cycles will be initiated when there is at least two consecutive rises in PSA at least 2 weeks apart. A three-week lead in with IL-2 should be sufficient to significantly increase the NK cell population. The dose of deJ591 is based on preliminary pharmacokinetic data from the Phase I ¹¹¹In-labeled deJ591 trial. The dose of antibody will be adjusted based on additional data analysis of patients treated in this manner.

[00426] A single cycle of treatment has been initiated. Patients who progress by radiographic documentation after one cycle will be removed from the study. Patients who responded by radiographic documentation, or who had stable disease by imaging with >50% decline in PSA value, or who had stable disease by imaging with stable PSA levels, will be examined every 3-4 weeks. Responding or stable patients will be eligible for re-treatment (a second 8-week cycle) following 2 consecutive PSA rises at least 2 weeks apart, at the discretion of the Principal Investigator and the option of the patient. Patients who have stable disease by imaging with a rising PSA (>25% of pre-treatment value) will be examined every 3-4 weeks. Re-treatment will be at the discretion of the Principal Investigator. In order to be retreated, the patient must have a immune reaction titer <1/100 and satisfy all initial Eligibility and Exclusion criteria. Re-treatment will be at the identical dose formulation as the initial dose given to the patient.

Patient Selection:

[00427] The trial is a pilot study to ascertain if low-dose IL-2 in combination with deJ591 has activity in prostate cancer. It is possible that low-dose IL-2 may have activity in prostate cancer patients. However, it has been decided not to treat patients with IL-2 alone, or delay antibody therapy, if the PSA declines after 3 weeks of therapy on low-dose IL-2, as it will be interesting to study the combined effect of deJ591 with IL-2. If significant activity is observed, a subsequent randomized trial of IL-2 alone vs. IL-2 with deJ591 will be conducted. It is unclear which stage of patient with prostate cancer might benefit from this approach. Therefore, at least ten patients will be treated, each in the following subgroups: 1) Biochemical relapse, hormone naive: rising PSA following radical prostatectomy or radiation therapy, without evidence of metastatic disease; 2) Biochemical relapse, hormone refractory: rising PSA following hormonal therapy without prior chemotherapy (these patients may or may not have radiographic documented metastatic disease); and 3) Hormone refractory, having received prior chemotherapy. About 30-40 patients will be enrolled on this trial.

Toxicity:

[00428] Toxicity will be scored using the Cancer Therapy Evaluation Program Common Toxicity Criteria, Version 2.0 (April 1999).

IL-2 Dosage modifications:

[00429] IL-2 will be permanently discontinued for any study drug-related Grade 4 toxicity except hematologic (which can be corrected with EPO or G-CSF or blood transfusions). If at any time during the study, ALT is ≥ 20 times the upper limit of normal, both IL-2 and mAb therapy will be permanently discontinued in that subject. Electrolyte abnormalities that can be readily corrected will not require permanent drug discontinuation.

[00430] This protocol allows for one dose level reduction of IL-2 of 25% to 0.9 mU/M^2 . Subjects receiving IL-2 may have their IL-2 interrupted for \geq Grade 1 toxicity for 24-48 hours. Toxicities that may cause IL-2 to be temporarily interrupted are:

Electrolyte abnormalities Grade 1 or higher that cannot be rapidly corrected;
Grade 1 or higher respiratory toxicity;

Grade 3 local reaction or any local reaction involving ulceration;
Fever > 38 °C, or intolerable flu-like symptoms or rigors;
Other Grade 3 or greater toxicity, either related or unrelated to IL-2;
Fever suspected to be related to an opportunistic infection;
Grade 1 fatigue; and
Grade 2 hematologic toxicity that can be corrected with EPO, G-CSF, or blood transfusions.

[00431] Subjects who have their IL-2 interrupted can have it resumed at the same dose or at one dose level reduction within 24-48 hours of stopping drug. The dose of IL-2 for that subject may later be increased to the initial dose at the discretion of the subject and the local investigator.

MAB deJ591:

[00432] Allergic events will be managed as follows: rash, pruritis, urticaria and wheezing will be treated with benadryl and/or steroids as clinically appropriate. Anaphylaxis or anaphylactoid signs or symptoms will be treated with steroids and/or epinephrine as clinically indicated. Patients will be treated in a general clinical research center equipped for cardiopulmonary resuscitation.

[00433] Both drugs will be discontinued in patients who experience any grade 3 or 4 toxicity during the three weeks when mAb is administered. Treatment with mAb will resume at a 25% reduction in dose of mAb deJ591 after the toxicity has returned to grade I or less. If grade 3 or 4 toxicity recurs on attenuated doses, mAb treatment will be discontinued. IL-2 treatment will continue for completion of the 8-week cycle.

Criteria for therapeutic response:

[00434] Response will be assessed either biochemically (PSA change) and/or by change in size of a measurable lesion/s using standard response criteria for prostate cancer (Dawson N.A. (1999) *Semin Oncol* 26:174-184; Bubley, G.J., et al. (1999) *J Clin Oncol*, 17:3461-3467)

[00435] Biochemical (PSA) response will be determined as described above. Criteria for measuring the disease is as follows: Complete response is defined as complete disappearance of

Statistical Methods:

[00439] For all outcome variable with measurements taken at various time points, a Repeated Measures Analysis of Variance (RMANOVA) will be carried out to determine any patterns of change over time. Upon finding a significant time effect, Bonferroni-adjusted pairwise contrasts will be calculated to determine which time points differ from one another. To simplify the analysis for data obtained at multiple time points, one may choose to "aggregate" the measurement values taken over time. An area under the curve (AUC) analysis for the PSA levels can be carried out. Alternatively, a "slope analysis" may be carried out.

Current Status of Results:

[00440] Six patients have been entered and three have completed 8 weeks of therapy; two patients are being retreated based on PSA stabilization. A third patient had objective disease progression. Toxicities have been expected and minor including fatigue, injection site reactions and asymptomatic thyroid function abnormalities. IL-2 mediated immune modulation is being evaluated by flow cytometry on peripheral blood to quantify expression of lymphocyte subsets pre- and post-mAb treatment.

Example 15: Conjugation of deJ591 to the maytansinoid cytotoxin DM1

[00441] This example describes a process for the production of the deJ591-DM1 immunoconjugate. The process is based on standard methods known in the art and can therefore be generalized to other antibodies, including other antibodies of the invention such as deJ415.

[00442] The methods of conjugation are based on several small scale experiments, including one experiment performed using 5g of deJ591 starting material (Lot 1552-60S) and three experiments performed using between 6.7g and 7.3g of deJ591 starting material (Lots 1552-168, 1552-104, and 1610-036).

[00443] The steps involved in the methods of conjugation are as follows:

1) 5g to 7.5g of deJ591 antibody is concentrated by tangential flow filtration (10kD NMWCO membranes) to 25-30 mg/ml and diafiltered against 5 volumes of 50mM potassium phosphate, 2mM EDTA, pH 6.0. The yield is typically between 98% and 100%.

2) The concentrated antibody is filtered through a 0.2 μ filter, if opalescent, and then modified with N-succinimidyl 4-(2-pyridyldithio)propionate (SPP) at a concentration of 20-22 mg/ml antibody and 7 molecules of SPP per molecule of antibody. The modification is

done in 50mM potassium phosphate, 2mM EDTA, 5% ethanol, pH 6.0, for 2.5 +/- 0.5 hours. The modification vessel is a 500ml round bottom flask.

3) The modified antibody is separated from the reaction mixture of step 2) using gel filtration chromatography and a Sephadex G-25™ column. The column load represents about 25% of the column volume and the chromatography is done in 50mM potassium phosphate, 2mM EDTA, pH 6.0, at a flow rate of 50 cm/hr. The modified antibody elutes between 38-75% column volume. Typically the yield of this step between 95% and 100% and the SPP to antibody ration is about 5.4 to 5.9 SPP molecule/antibody.

4) At a concentration of about 10mg/ml, the modified antibody is conjugated with DM1 (using 1.7 molecules of DM1/molecule of SPP conjugated to the antibodies) for 20 +/- 4 hours. Typically, the reaction time is between 16.25 and 17.7 hours and is carried out in a 1L round bottom glass flask equipped with a magnetic stirring bar. The conjugation reaction is done in 3% DMA, 10% sucrose (100mg sucrose/ml of reaction). At the end of the reaction the conjugated antibody is filtered through a 2.0 µ filter and a spectrophotometric reading is taken.

5) The conjugated antibody is separated from unreacted DM1 by gel filtration chromatography using a Sephadex G-25™ column. The column load represents 22-23% of the column volume and the flow rate is about 50 cm/hr. The column is equilibrated and run in 20mM succinate, 5% sucrose (50mg/ml), pH 5.5. The antibody conjugate elutes between about 31% and 65% of column volume, and is collected from the start of the peak elution to the start of the peak trailing edge as a single fraction, followed by fractionation of the remaining peak material in 15x2% column volume fractions. All fractions are adjusted to 100mg/ml of sucrose (10% sucrose) through the addition of appropriate amounts of 50% sucrose. The 2% column volume fractions are assayed by analytical sizing (TSK 3000SWL) and selected fractions (fractions 1 and 2) are pooled together with the main peak. The fractions are assayed using analytical sizing with the pooling criterion being the 24 minute peak representing <20% or the total peak area. Typically the yield of this step is between 60% and 65% with the exception of run 1552-104 where there was no sucrose present in the reaction and/or purification mixture. The eluted antibody concentration ranges from 3.8 to 4.2 mg/ml and the ration of DM1/antibody ranges from 3.6 to 3.9.

6) The antibody conjugate is then concentrated to 7-10 mg/ml using a 10kD NMWCO tangential flow filtration membrane and diafiltered against 5 volumes of 50mM

succinate, 10% sucrose, pH 5.5 (Inlet Pressure < 10 psi). Following diafiltration the antibody conjugate is adjusted to 5 mg/ml. Typical yield for this step is between 92% and 100%, with the final protein concentration being between 4.85 and 5.1 mg/ml.

7) Finally, the antibody conjugate is filtered through a 0.2 m filter and aliquoted to the specified volumes. Step yield is between 90% and 100% and the final DM1-antibody ratio is 3.5 to 3.8.

[00444] The resulting deJ591-DM1 conjugates were analyzed according to appearance, concentration, DM1/antibody ratio, endotoxin, non-specific cytotoxicity, acetone extractable DM1, analytical sizing, reduced and non-reduced SDS-PAGE, pH, bioburden, specific cytotoxicity, and IEF. Selected analytical results for lots 1552-168, 1552-104, and 1552-036 are shown in Table 19, below.

Table 19

Lot No.	Amount Recovered	Concentration (mg/ml)	DM1/antibody	Process Recovery (%)
1552-168	3.85	5.1	3.7	57.2
1552-104	2.75	4.85	3.5	37.8*
1610-036	3.41	5.05	3.8	47.2
Mean	3.34	5.00	3.67	47.4
Standard Dev.	0.55	0.13	0.15	9.7
% c.v.	16.6	2.6	4.2	20.5

* Lower recovery due partly to the lack of sucrose in the conjugation reaction and the second G-25 gel filtration run and partly to the fact that the front end of the product peak was not collected due to a malfunction in the chart recorder.

Example 16: Use of the mAbs for targeted delivery of cytotoxic drugs to prostate cancer cells

[00445] Anti-PSMA antibodies can be conjugated to substances with high cytotoxic potential, such as drugs of the maytansinoid class. Maytansinoids exert their cytotoxic effects by interfering with the formation and stabilization of microtubules. They have 100- to 1000-fold greater cytotoxic potential than conventional chemotherapeutic agents (such as doxorubicin, methotrexate, and Vinca alkaloids) (Chari, R.V.J. et al. (1992) *Cancer Res.* 52: 127).

[00446] Both murine and deimmunized J591 antibodies have been conjugated to the maytansinoid, DM1, via a hindered disulfide bond. This bond is cleaved intracellularly allowing release of the drug. One or more lysine residues in the constant regions of the antibodies were conjugated to a linker containing a pyridyldithio group, which was, in turn, coupled to a maytansinoid toxin. A ratio of 3 to 4 moles of maytansinoid per mole of IgG is preferred.

[00447] The process for the DM1-linked J591 antibodies starts by reacting J591 with a linker that contains both a pyridyldithio group and a N-hydroxysuccinimide leaving group. In this case, the linker was N-succinimidyl 4-(2-pyridyldithio)propionate (or SPP), although other linkers can be used. The products of the reaction include modified J591 antibodies that contain one or more linker groups (4-(2-pyridyldithio)propionate) attached to surface exposed lysine groups, with the linker groups retaining the pyridyldithio reactive groups, and N-hydroxysuccinimide leaving groups. The J591 antibodies are then separated from the reaction mixture and N-hydroxysuccinimide by gel filtration, e.g., using sephadex G25. The modified J591 antibodies are reacted with DM1, which contains a thiol group that reacts with the pyridyldithio groups now present on the surface of the modified antibody, thereby producing J591-DM1 immunoconjugates and thiopyridine. The J591-DM1 immunoconjugate is isolated from the reaction mixture and thiopyridine by size exclusion chromatography, e.g., using a sephacryl S300 column. Methods for preparing maytansinoid conjugates are described in US Patent Nos. 5,208,020; 5,475,092; 5,585,499; 5,846,545; and 6,333,410, the contents of which are incorporated by reference.

[00448] A study evaluating the efficacy, toxicity, and antigen selectivity of the murine J591-DM1 immunoconjugate in the treatment of prostate cancer cells *in vivo* is described below.

Experiment 1: Establish Efficacy, Toxicity, and Dose-Response Curves In-Vivo

[00449] Tumor xenografts of LNCaP cells were established in the right flank of BALB/c mice (5×10^6 cells injected subcutaneously). Animals were observed until tumors were visible (7 - 10 mm). Animals were then treated with PBS, unconjugated mAb J591, unconjugated DM1, both mAb J591 and DM1 but unconjugated, and J591-DM1 immunoconjugate (100, 200, 300, and 400 mcg/day intraperitoneal qday x 5 days). The mAb J591 was modified to introduce dithiopyridyl groups and then conjugated to DM1 via a hindered disulfide bond, as described above. Unconjugated mAb J591 and DM1 were given in equimolar concentrations.

Experiment 2: Selectivity for PSMA-positive tumors

[00450] Tumor xenografts of both LNCaP cells (5×10^6 cells injected subcutaneously in the right flank) and PC3 cells (3×10^6 cells injected subcutaneously in the left flank) were established in BALB/c mice. Animals were observed until LNCaP tumors were visible (PC3 tumors were present but much smaller). Animals treated with J591-DM1 immunoconjugate: 300 mcg/day intraperitoneal qday x 5 days

Experiment 3: Determine optimal dosing schedule for the immunoconjugate

[00451] Tumor xenografts of LNCaP cells were established subcutaneously in the right flank of BALB/c mice. When tumors were visible, animals were treated with PBS, unconjugated mAb J591, J591-DM1 immunoconjugate (300 mcg/day intravenously qday x 5 days; one course given), J591-DM1 immunoconjugate (400 mcg/day intravenously every other day for 5 doses in 10 days; one course given), and J591-DM1 immunoconjugate (300 mcg/day intravenously qday x 5 days; two courses given for a total of 10 doses). For animals receiving 2 courses of J591-DM1 immunoconjugate, the second 5-day course was given at tumor volume nadir (typically seen on days 21 - 24 after completion of the first course).

[00452] All animals were photographed pre- and post-treatment and tagged for identification. Tumor volume was determined (length x width x depth) and closely followed. Animal weight was followed as a measure of toxicity.

Results: Experiment 1:

[00453] All mice treated with unconjugated DM1 (either alone or with unconjugated mAb J591) died within 48 hours of treatment completion. Immunoconjugate at doses of 400 mcg/day was lethal to 50% of the mice. With doses of 300 mcg/day, significant reductions in tumor volume were noted with several complete responses and minimal toxicity. These complete responses were not durable, however, with subsequent increases in tumor size noted 2 - 20 days after tumor volume nadir.

Results: Experiment 2:

[00454] In mice with both LNCaP and PC3 tumors, LNCaP tumor volume decreased substantially after treatment with the J591-DM1 immunoconjugate. Although complete responses were not achieved in these animals, tumor volume nadirs averaged 0.08 cm³. PC3 tumor growth was not affected by the J591-DM1 immunoconjugate as noted by a steady increase in PC3 tumor volume after treatment.

Experiment 3:

[00455] Although route of administration was different (intravenous vs. intraperitoneal), treatment with one course of J591-DM1 immunoconjugate paralleled the results obtained from Experiment 1. Complete responses were achieved (at days 21 - 24) but these results were not durable. In animals treated with every other day dosing of 400 mcg, toxicity was demonstrated (weight loss > 10 % of total body weight) but all animals eventually survived. Tumor volume decreased in all animals but complete responses were not achieved. All animals treated with 2 courses of J591-DM1 immunoconjugate have achieved complete responses lasting at least through day 66.

Conclusions:

[00456] Murine J591 anti-PSMA antibody can be conjugated effectively to drugs of the maytansinoid class (such as DM1). These J591-DM1 immunoconjugates provide highly selective, antigen-specific targeted delivery of this cytotoxic drug to PSMA-positive prostate cancer cells in-vivo. The greatest reduction in tumor volume with minimal toxicity was noted at a dose of 300 mcg/day. The optimal dosing schedule appears to be two 5-day courses of J591-DM1 immunoconjugate with the second course given at tumor volume nadir (day 21 - 24).

Example 17 – Pharmacodynamics and Efficacy of the deJ591-DM1 immunoconjugate

[00457] In parallel to the experiments described in Example 16, additional experiments were performed with the deJ591-DM1 immunoconjugate. The experiments and results obtained therefrom are described below.

In vitro pharmacodynamics of deJ591-DM1 and DM1:

[00458] Experiment: LNCaP cells were plated into 96 well plates at an initial density of 1000 cells per well for a proliferation assay. deJ591-DM1 or DM1 was added to the wells over a range of concentrations (0.01 nM to 100 nM) and left in contact with the cells for defined periods of time (0.5, 2, 8, 24, 72 and 144 hours). At the end of that period the drug containing media was removed and replaced with drug free media until a total of 144 hours elapsed from the start of the experiment. The resulting effect of drug exposure on proliferation was then determined.

[00459] Results: The pharmacodynamic analysis using the methodology developed by Kalns et al. (1995), Cancer Research 55:5315-5322, the contents of which are incorporated herein by reference, indicates that when considering the relative importance to observed effect from the variables (1) time of exposure and (2) concentration, that exposure time is more important for deJ591-DM1 compared to DM1, where concentration is the more important variable.

Serum levels of deJ591-DM1 in mice after a single IV dose

[00460] Experiment: Mice were administered an intravenous 14.5 mg/kg dose of deJ591-DM1 and sera samples collected for analysis of the test article using an ELISA assay for human antibody. Samples were collected from groups of 3 mice at 6, 24, 48, 72 and 168 hours. Data were fit to a bi-exponential equation to determine pharmacokinetic parameters. These parameters were used to simulate serum levels after multiple dosing.

[00461] Results: Analysis of the pharmacokinetic data indicated a serum half life of deJ591-DM1 in mice as measured in the ELISA assay, to be 130 hours.

Effect of dose interval on efficacy against CWR22 prostate xenografts

[00462] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm³ size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only (every 7 days) or deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg DM1) given on one of the following schedules (every 7, 14, 21, or 28 days). All treatments were given intravenously. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00463] Results: Differences in the schedule of administration have distinct effects on the observed tumor growth for the CWR22 xenograft in SCID mice. The tumor growth can be characterized as slowed growth for the schedule of 21 or 28 day dosing interval until they reached the maximum size permitted under our IACUC regulations. For the 7 and 14 day dosing interval schedule, there is an apparent block in tumor growth with a resumption of normal growth kinetics approximately 30 days after the last dose. The results when considered with the pharmacokinetic simulation suggest a relationship between duration of exposure and maintaining a minimum effective concentration.

Efficacy of deJ591-DM1 compared to the unconjugated antibody (deJ591) or the unconjugated tumor inhibitory agent (DM1)

[00464] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm³ size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only, deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg DM1), deJ591 at the same dose as deJ591-DM1, or DM1 given at a dose of 240 ug/kg. All treatments were given intravenously and on a schedule of every three days for 5 doses. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00465] Results: The unconjugated anti-PSMA antibody (deJ591) had no significant effect on reduction in the rate or extent of CWR22 xenograft tumor growth. DM1 administered as free drug produced some tumor growth delay, but was a minor response compared to the deJ591-DM1 administered on the same schedule with the same molar equivalent of the active DM1. The deJ591-DM1 produced a suppression of tumor growth for approximately 20 days following the last administered dose.

Efficacy of DEJ591-DM1 at different doses

[00466] Experiment: Male SCID mice were implanted by serial passage of CWR22 prostate tumor xenograft. When these tumors reached 200 – 250 mm³ size (estimated from external caliper measurement), mice were randomized into treatment groups of 8 to receive vehicle only, deJ591-DM1 at a dose of 14.5 mg/kg antibody conjugate (equivalent to 240 ug/kg

DM1), or a lower dose of 7.25 mg/kg. All treatments were given intravenously and on a schedule of every seven days for 5 doses. Tumor growth and animal health were continually monitored throughout the study with tumor growth measured every 3 days.

[00467] Results: A dose response relationship is evident for the CWR22 xenograft tumor growth inhibition by deJ591-DM1. This is shown on a 7 day dosing interval study for 2 different doses of deJ591-DM1. At the higher dose, there is suppression of tumor growth with some reduction from the initial tumor volume. At the lower dose there is not the same reduction from initial tumor volume and there is a more rapid return to normal growth kinetics of approximately 10 days following the last dose administered compared to the higher dose.

Example 18 – Bone marrow involvement in advanced prostate cancer patients demonstrated on bone marrow biopsy

[00468] Bone marrow involvement in advanced prostate cancer is not routinely examined. We report on the results of bone marrow biopsies performed on advanced, hormone-refractory prostate cancer patients.

[00469] Screening diagnostic studies were performed on hormone-refractory prostate cancer patients to determine eligibility for two phase I radioimmunotherapy clinical trials. Studies included serologic testing, bone scan, CT scans of the head, chest, abdomen and pelvis, and a bone marrow biopsy taken from the iliac crest. A total of Thirty-nine patients have been screened thus far.

[00470] All patients had advanced disease as determined by bony or soft tissue metastases on imaging and/or three consecutive rises in serum PSA levels. All patients had received prior hormonal therapy, and the majority had received local therapy, including radical prostatectomy (N=15), radiotherapy (N=19), and/or chemotherapy (N=19). Sixteen patients (41%) had histologic evidence of metastatic prostate cancer on bone marrow biopsy. Of the thirty-nine screened patients, thirteen (33%) had significant bone marrow involvement (>10% involvement), making them ineligible for entry into these clinical trials.

[00471] Patients with bone marrow involvement had significantly higher serum alkaline phosphatase (ALP) levels (median 374 U/L vs. 96 U/L, $p<0.001$) and significantly lower serum hemoglobin (median 11.6 g/dL vs. 12.7 g/dL, $p=0.02$). There was no difference in serum hemoglobin between patients with prior chemotherapy and/or prior radiotherapy. When the ALP

was normal (<120 U/L) or elevated (>120 U/L,) 0% and 75% of patients, respectively, had metastatic prostate cancer on bone marrow biopsy ($p<0.0001$). Patients with bone scans indicating bony metastases in three or more different anatomic sites (spine, thorax, pelvis, appendicular or calvarium) vs. two or less sites, were more likely to have bone marrow involvement (54% vs. 9%, $p=0.01$). Age, initial Gleason sum, serum PSA, PSA doubling time, the presence of soft-tissue metastases, prior local treatment or chemotherapy, or other hematologic parameters (WBC, platelet count) were not significantly different between the two groups.

[00472] Bone marrow involvement in advanced prostate cancer patients is not routinely examined, but is present in a large minority of cases. Current clinical staging studies may significantly underestimate bone marrow involvement, although elevation of alkaline phosphatase or depressed serum hemoglobin may be correlative. This situation should be considered in clinical trials carrying potential hematologic toxicity.

Example 19: A Novel Sandwich Enzyme-Linked Immunoassay (ELISA) for Quantification of Prostate-Specific Membrane Antigen

[00473] Ideally, serum PSMA should be detectable with a simple, rapid, reproducible and quantitative ELISA assay. Our objective was to establish an ELISA assay to measure serum PSMA.

[00474] Ninety-six well plates were coated with an anti-PSMA antibody as a "capture" antibody. Dilutions of serum from males and females "spiked" with recombinant PSMA (rPSMA), semen, LNCaP lysates, as well as the standard (rPSMA range 1.6-1600 ng/ml) were then added to these wells. A non-competing biotinylated anti-PSMA antibody (that recognizes a different epitope on PSMA) was then added as the "detection" antibody. Avidin phosphatase followed by p-nitrophenyl phosphate (substrate) was added. Optical densities were then measured.

[00475] The standard curve for the assay was linear through a range of 5-1600 ng/ml (correlation coefficient $> .99$). Using this assay, PSMA was detected in LNCaP lysate, semen, and "spiked" serum.

Equivalents

[00476] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A modified anti-prostate specific membrane antigen (PSMA) immunoglobulin, comprising the amino acid sequence shown as SEQ ID NO:22 or SEQ ID NO:50, or the light chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.
2. The modified PSMA immunoglobulin of claim 1, which is encoded by the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.
3. A modified anti-PSMA immunoglobulin, comprising a heavy chain variable region, comprising the amino acid sequence shown as SEQ ID NO:21 or SEQ ID NO:49, or the heavy chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.
4. The modified anti-PSMA immunoglobulin of claim 3, which is encoded by the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.
5. A modified anti-PSMA antibody, or an antigen-binding fragment thereof, comprising:
a light chain variable region comprising the amino acid sequence shown as SEQ ID NO:22 or SEQ ID NO:50, or the light chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and
a heavy chain variable region comprising the amino acid sequence shown as SEQ ID NO:21 or SEQ ID NO:49, or the heavy chain variable region amino acid sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

6. The modified antibody, or fragment thereof, of claim 5, which comprises two heavy chains and two light chains.
7. The modified antibody, or fragment thereof, of claim 5, wherein the heavy chain constant region is of human isotype IgG1.
8. A pharmaceutical composition comprising the modified antibody, or fragment thereof, of claim 5, and a pharmaceutically acceptable carrier.
9. The modified antibody, or fragment thereof, of claim 5, further comprising a cytotoxic moiety or a detectable label.
10. The modified antibody, or fragment thereof, of claim 9, wherein the cytotoxic moiety is a cytotoxin, a therapeutic agent or a radioactive ion.
11. The modified antibody, or fragment thereof, of claim 10, wherein the radioactive ion is iodine (^{131}I), indium (^{111}In), yttrium (^{90}Y) or lutetium (^{177}Lu).
12. The modified antibody, or fragment thereof, of claim 10, wherein the cytotoxic moiety is maytansinoid.
13. The modified antibody, or fragment thereof, of claim 12, wherein the maytansinoid is maytansinol.
14. A nucleic acid sequence having a nucleotide sequence encoding a modified anti-PSMA immunoglobulin, comprising a light chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

15. A nucleic acid sequence having a nucleotide sequence encoding a modified anti-PSMA immunoglobulin, comprising a heavy chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

16. A first and second nucleic acid sequences having nucleotide sequences encoding light and heavy chain variable regions, respectively, of a modified anti-PSMA antibody or an antigen binding fragment thereof, wherein the light chain variable region comprises the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and the heavy chain variable region comprises the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:2, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174.

17. A method of producing a modified anti-PSMA antibody, or antigen binding fragment thereof, comprising:

providing a first nucleic acid encoding a light chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-581 of SEQ ID NO:25, SEQ ID NO:52, or the light chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174;

providing a second nucleic acid encoding a heavy chain variable region comprising the nucleotide sequence shown as nucleic acid residues 261-605 of SEQ ID NO:23, SEQ ID NO:51, or the heavy chain variable region nucleotide sequence of the antibody produced by the NS0 cell line having ATCC Accession Number PTA-3709 or PTA-4174; and

introducing said first and second nucleic acids into a host cell under conditions that allow expression and assembly of said light and heavy chain variable regions.

18. The method of claim 17, wherein the first and second nucleic acids are linked.

19. The method of claim 17, wherein the first and second nucleic acids are unlinked.
20. The method of claim 17, wherein the host cell is a eukaryotic or prokaryotic cell.
21. The method of claim 20, wherein the host cell is a mammalian cell.
22. The method of claim 21, wherein the mammalian cell is selected from the group consisting of a lymphocytic cell line, NS0 cell, CHO cell, COS cell, and a cell from a transgenic animal.
23. A method of ablating or killing a prostatic cell or a cancer cell, comprising contacting the cell, or a vascular endothelial cell proximate to cell, with the modified antibody, or fragment thereof of claim 5, such that the cell is ablated or killed upon binding of the antibody to the cell or the vascular endothelial cell proximate thereto.
24. A method of treating, or preventing, a prostatic or cancerous disorder, in a subject, comprising administering to the subject the modified antibody molecule of claim 5, in an amount effective to treat or prevent the disorder.
25. The method of claim 23 or 24, wherein the modified antibody, or fragment thereof, is associated with a cytotoxic agent or moiety.
26. The method of claim 25, wherein the cytotoxic agent or moiety is selected from the group consisting of an antimetabolite, an alkylating agent, cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines, and an anti-mitotic agent.
27. The method of claim 24, wherein modified antibody, or fragment thereof, is administered in combination with a cytotoxic agent.

28. The method of claim 25, wherein modified antibody, or fragment is coupled to a cytotoxic moiety.

29. The method of claim 28, wherein the cytotoxic moiety is a cytotoxin, a therapeutic agent or a radioactive isotope.

30. The method of claim 29, wherein the radioactive isotope is iodine (^{131}I), yttrium (^{90}Y) or lutetium (^{177}Lu).

31. The method of claim 29, wherein the cytotoxic moiety is maytansinoid.

32. The method of claim 31, wherein the maytansinoid is maytansinol.

33. The method of claim 31, wherein the maytansinoid is DM1.

34. The method of claims 23, wherein the cell is a malignant, normal, benign or hyperplastic, prostate epithelial cells.

35. The method of claim 23, wherein the cell is a renal, an urothelial, colon, rectal, lung, breast or liver, cancerous or metastatic cell.

36. The method of claim 24, wherein the disorder is selected from the group consisting of genitourinary inflammation, prostatitis, benign enlargement, prostatic cancer and testicular cancer.

37. The method of claim 24, wherein the disorder is selected from the group consisting of solid tumors, soft tissue tumors, and metastatic lesions.

38. The method of claim 37, wherein the solid tumors are malignancies selected from the group consisting of sarcomas, adenocarcinomas, and carcinomas.

39. The method of claim 37, wherein the disorder is a malignancy affecting lung, breast, lymphoid tissue, gastrointestinal tissue, colon, rectum, genitourinary tract, renal cells, urothelial cells, pharynx, bladder, liver, lung, small intestine and esophagus.

40. A method for detecting the presence of a PSMA protein, in a sample comprising prostatic or cancerous cells, *in vitro*, comprising: (i) contacting the sample or a reference sample with the modified anti-PSMA antibody, or fragment thereof, of claim 7, under conditions that allow interaction of the anti-PSMA antibody and the PSMA protein to occur; and (ii) detecting formation of a complex between the anti-PSMA antibody, and the sample or the reference sample, wherein a statistically significant change in the formation of the complex in the sample relative to a reference sample is indicative of the presence of PSMA in the sample.

41. A method for detecting the presence of PSMA *in vivo*, comprising: (i) administering to a subject the modified anti-PSMA antibody, or antigen binding fragment thereof, of claim 7, in detectably labeled form, under conditions that allow interaction of the modified anti-PSMA antibody, or fragment thereof, and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA, wherein a statistically significant change in the formation of the complex in the subject relative to a reference is indicative of the presence of the PSMA.

42. A method of diagnosing or staging, a prostatic or cancerous disorder, comprising: (i) identifying a subject having, or at risk of having, the disorder; (ii) obtaining a sample of a tissue or cell affected with the disorder; (iii) contacting said sample or a control sample with the modified anti-PSMA antibody or fragment thereof of claim 7, under conditions that allow interaction of the binding agent and the PSMA protein to occur, and (iv) detecting formation of a complex, wherein a statistically significant increase in the formation of the complex between the antibody or fragment thereof with respect to the control sample, is indicative of the disorder or the stage of the disorder.

43. A method for *in vivo* imaging PSMA-expressing tumors comprising: (i) administering to a subject, or a control subject a labeled modified anti-PSMA antibody or

fragment thereof of claim 7, under conditions that allow interaction of the modified anti-PSMA antibody or fragment thereof and the PSMA protein to occur; and (ii) detecting formation of a complex between the antibody or fragment and PSMA.

44. A method of treating pain experienced by a subject having or diagnosed with benign prostatic hyperplasia or prostate cancer, or non-prostate cancer, comprising: administering an anti-PSMA antibody to a subject in an amount sufficient to treat the pain associated with prostate disease or non-prostate cancer.

45. A method for treating metastatic lesions associated with prostate cancer in a subject, comprising administering to the subject the modified antibody molecule of claim 5, in an amount effective to treat or prevent the disorder.

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Amino Acid Sequence of Murine J591 Heavy Chain (CDRs are marked,
numbering as Kabat)

EVQLQQSGPELKKPGTSVRISCKTS | GYTFTEYTIH | WVKQSHGKS
1 10 20 30 40
 CDR1

LEWIG | NINPNNGGTTYNQKFED | KATLTVDKSSSTAYMELRSLTS
 50 60 70 80
 CDR2

EDSAVYYCAA | GWNFDY | WGQGTTLTVSS
 90 100 110
 CDR3

FIG. 1A

Amino Acid Sequence of Murine J591 Light Chain (CDRs are marked,
numbering as Kabat)

DIVMTQSHKFMSTSVGDRVSIIC | KASQDVGTAVD | WYQQKPGQSP
1 10 20 30 40
 CDR1

KLLIY | WASTRHT | GVPDRFTGSGSGTDFTLTITNVQSEDLADYFC
 50 60 70 80
 CDR2

| QQYNSYPLT | FGAGTMLDLK
 90 100
 CDR3

FIG. 1B

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Amino Acid Sequence of DeImmunised J591 Heavy Chain (CDRs are marked, numbering as Kabat)

EVQLVQSGPEVKKPGATVKISCKTS | GYTFTEYTIH | WVKQAPGKG
1 10 20 30 40
CDR1

LEWIG | NINPNNGGTTYNQKFED | KATLTVDKSTDYAMELSSLRS
 50 60 70 80
CDR2

EDTAVYYCAA | GWNFDY | WGQGTLLTVSS
 90 100 110
CDR3

FIG. 2A

Amino Acid Sequence of DeImmunised J591 Light Chain (CDRs are marked, numbering as Kabat)

DIQMTQSPSSLSTSVGDRVTLTC | KASQDVGTAVD | WYQQKPGPSP
1 10 20 30 40
CDR1

KLLIY | WASTRHT | GIPSRFSGSGTDFTLTISSLQPEDFADYYC
 50 60 70 80
CDR2

| QQYNSYPLT | FGPGTKVDIK
 90 100
CDR3

FIG. 2B

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Location of T cell epitopes in J591 VH

	10	20		
1	E V Q L Q Q S G P E L V K P G T S V R I S C K T S		J591 MoVH	
1	E V Q L V Q S G P E V K K P G A T V K I S C K T S		J591 DIVH	
	30	40	50	
26	G Y T F T E Y T I H W V K Q S H G K S L E W I G N		J591 MoVH	
26	G Y T F T E Y T I H W V K Q A P G K G L E W I G N		J591 DIVH	
	60	70		
51	I N P N N G G T T Y N Q K F E D K A T L T V D K S		J591 MoVH	
51	I N P N N G G T T Y N Q K F E D K A T L T V D K S		J591 DIVH	
	80	90	100	
76	S S T A Y M E L R S L T S E D S A V Y Y C A A G W		J591 MoVH	
76	T D T A Y M E L S S L R S E D T A V Y Y C A A G W		J591 DIVH	
	110			
101	N F D Y W G Q G T T L T V S S		J591 MoVH	
101	N F D Y W G Q G T L L T V S S		J591 DIVH	

FIG. 3A

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Location of T cell epitopes in J591 VK

	10	20	
1	D I V M T Q S H K F M S T S V G D R V S I I C K A		J591 MoVK
1	D I Q M T Q S P S S L S T S V G D R V T L T C K A		J591 DIVK
	30	40	50
26	S Q D V G T A V D W Y Q Q K P G Q S P K L L I Y W		J591 MoVK
26	S Q D V G T A V D W Y Q Q K P G P S P K L L I Y W		J591 DIVK
	60	70	
51	A S T R H T G V P D R F T G S G S G T D F T L T I		J591 MoVK
51	A S T R H T G I P S R F S G S G S G T D F T L T I		J591 DIVK
	80	90	100
76	T N V Q S E D L A D Y F C Q Q Y N S Y P L T F G A		J591 MoVK
76	S S L Q P E D F A D Y Y C Q Q Y N S Y P L T F G P		J591 DIVK
	101		
101	G T M L D L K		J591 MoVK
101	G T K V D I K		J591 DIVK

FIG. 3B

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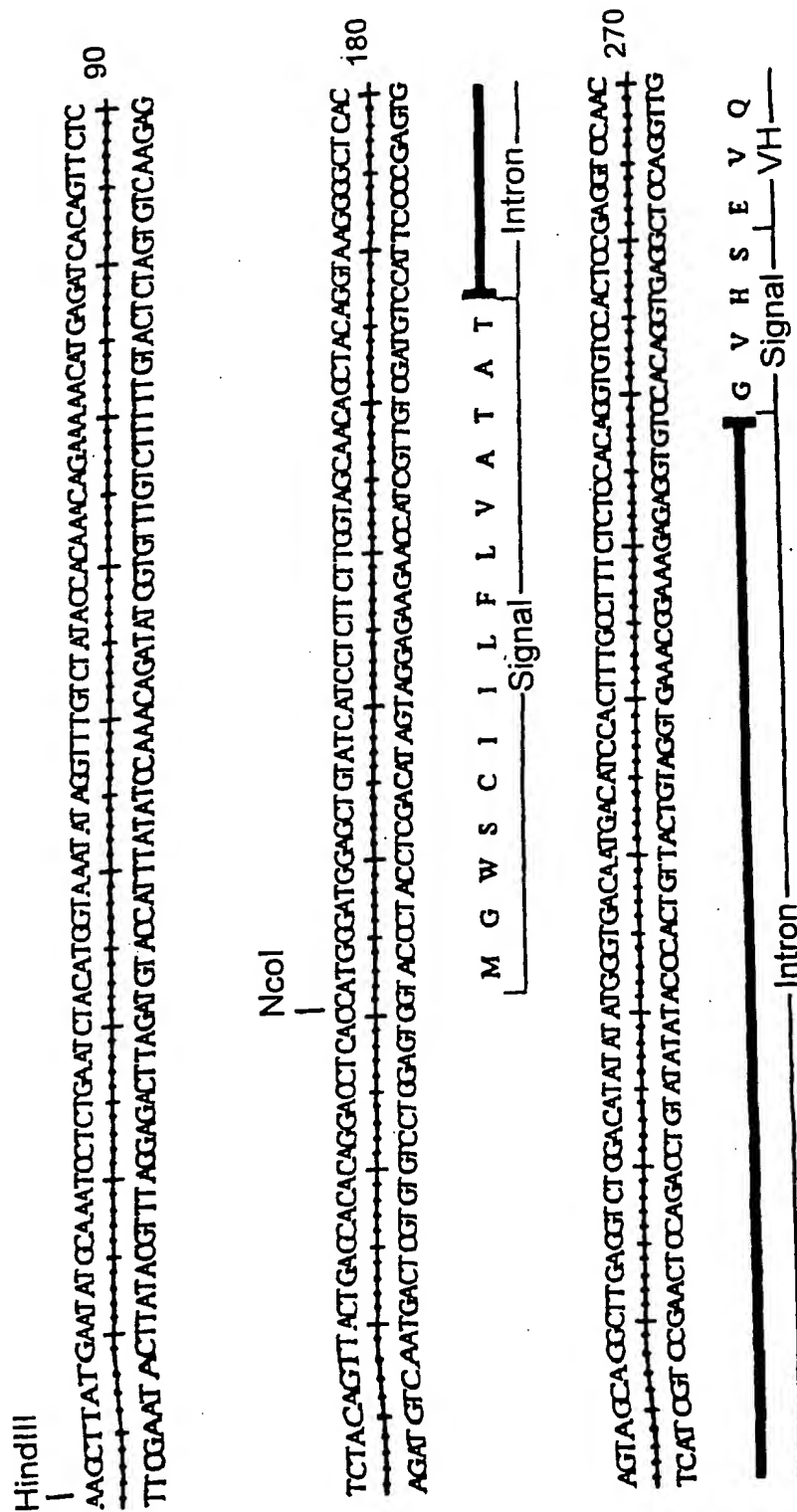


FIG. 4A-1

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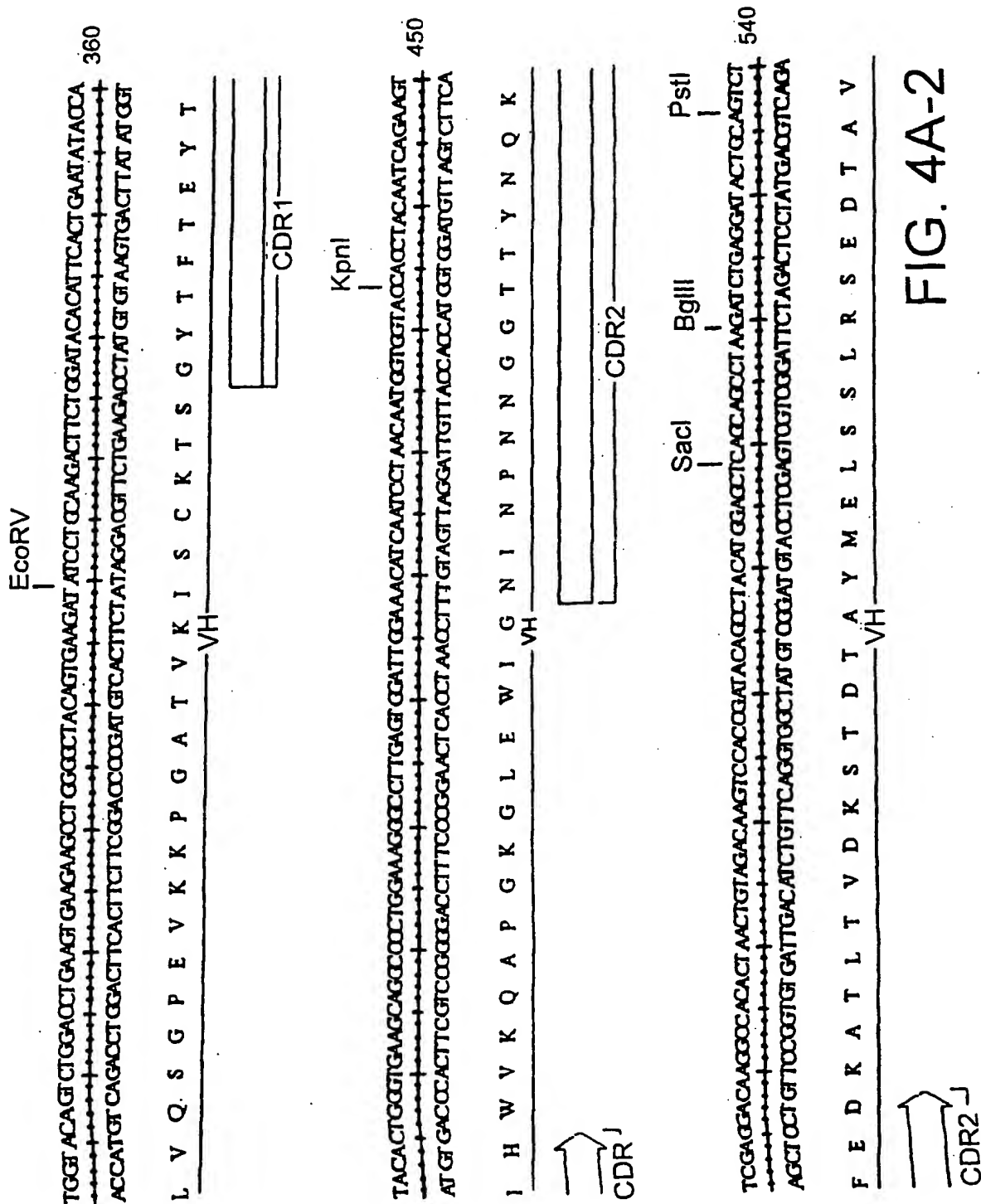


FIG. 4A-2

PvuII BsgI
| |
ATTATTGTGCAGCTGGTTGGAACCTTTTGACTACTGGGGCCAAAGCGAACCCTCCTCAOOGTCTCTCAGGTGAGTCCTTAGAACCTCTCTCTT
TAAT AACACGTGCAACCAACCTT GAACCTGTATGACCCC GTTCTCCTGGGAOCGAG CCCAGAGGAGTCCACT CAGGAAT GCT CGAGAGA GAA

630

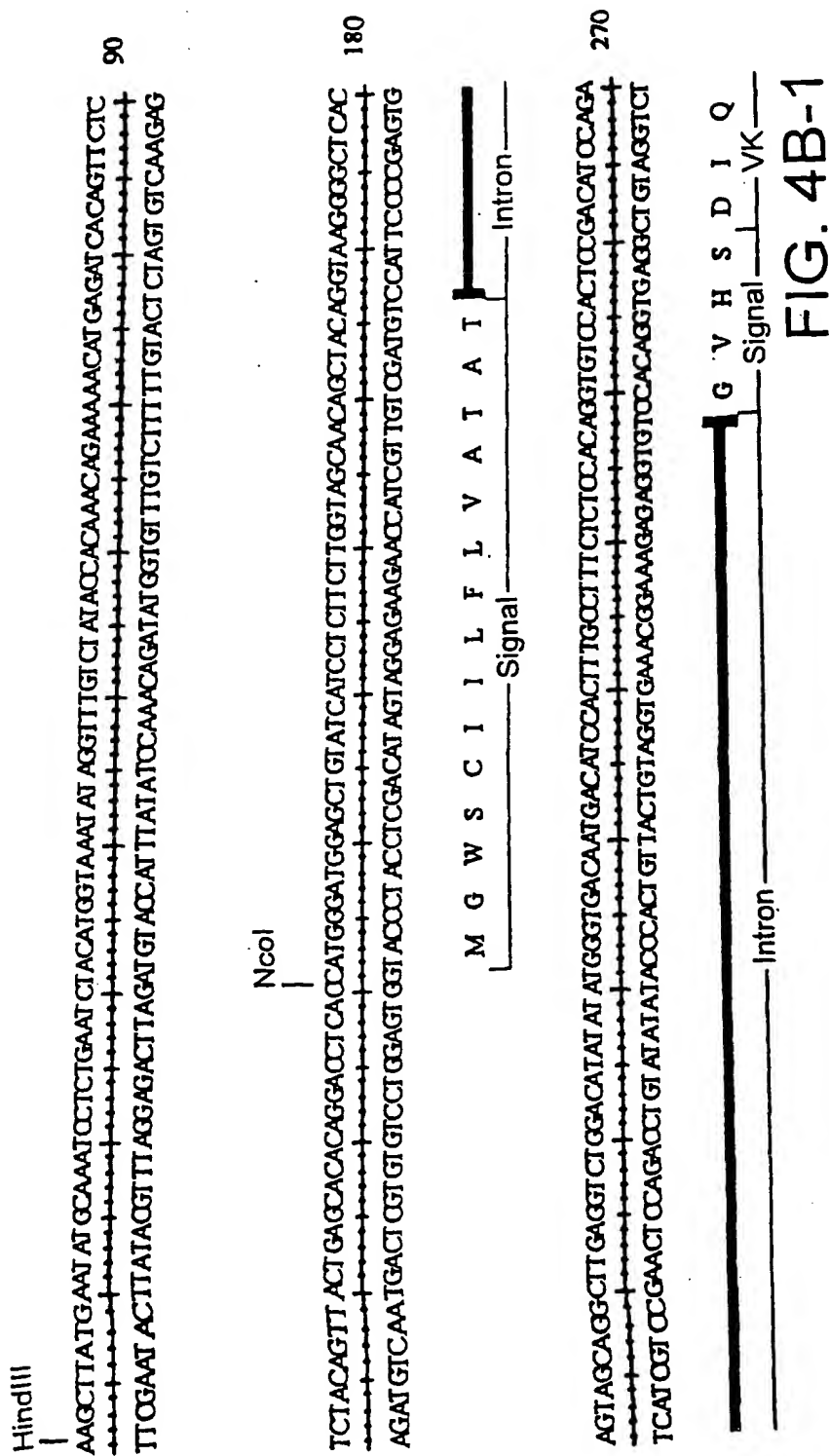
Y Y C A A G W N F D Y W G Q G T L L T V S S
VH

CT ATTCAACCTTAAATAGATTTTACTCCATTTTGTTGGGGGGAATGTGTGTATCTGAATTTCAAGTCAATGAAGGACTACGGACACCTTGG
GATAAGTCGAATTTATCTAAATGAGGTAAACAACCCGCCCTTTACACACATAGACTTAAAGTCCAGTACTTCTTCTGATCCCTGTGGAAAC

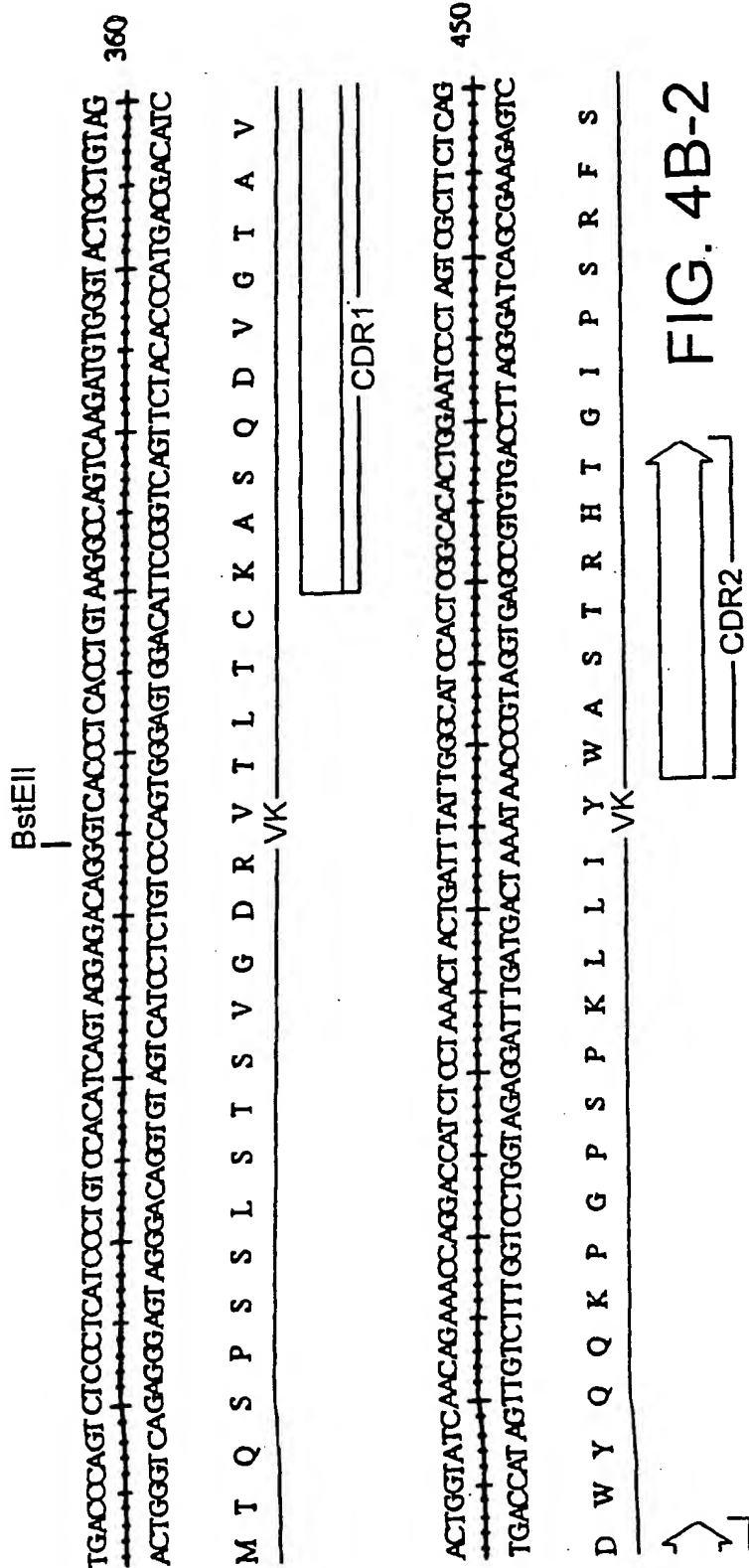
XmaI
 SmaI
 BamHI
 807

FIG 4A-3

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GCAGTGGATCTGGACAGACTTCACCTCAACATTCTCTAGCTTCAGCCTGAAGACTTTGCAGATTAATTACTGTCAGCAATATAACAGCT
 CGTCACCTAGACCTCTGTCTGAAGTGAGAGTGGTAAGATCAGAACTCGGACTTCTGAACGCTCTAAT AATGACAGTCGTT ATATTGTGGA
 540
 G S G S G T D F T L T I S S L Q P E D F A D Y Y C Q Q Y N S
 —VK—

—CDR3—

BamHI

ATCCTCTCAGGTCGGTCTGGGAACAAGGTGGACATCAACGCTGAGTAGAATTTAACTTTGCTTCCTCAGTTGGATCC
 TAGGAGAGTGCAAGCCAGGACCTGGTCCACCTGTAGTTGCACATCATCTTAAATTGAAACGAGGAGTCAACCTAGG
 620

Y P L T F G P G T K V D I K
 —VK—

—CDR3—

FIG. 4B-3

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		N I V M T Q F P K S M S A S A G E R M T L T C K A S E																										Majority	
		10													20														
1		N	I	V	M	T	Q	F	P	K	S	M	S	A	S	A	G	E	R	V	T	L	T	C	K	A	S	E	J415VK
1		N	I	V	M	T	Q	S	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK1
1		N	I	V	M	T	Q	S	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK2
1		N	I	V	M	T	Q	S	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK3
1		N	I	V	M	T	Q	S	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK4
1		N	I	V	M	T	Q	F	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK5
1		N	I	V	M	T	Q	F	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK6
1		N	I	V	M	T	Q	F	P	K	S	M	S	A	S	A	G	E	R	V	T	L	T	C	K	A	S	E	J415DIVK7
1		N	I	V	M	T	Q	F	P	K	S	M	S	A	S	A	G	E	R	M	T	L	T	C	K	A	S	E	J415DIVK8
		N V G T Y V S W Y Q Q K P T Q S P K M L I Y G A S N R																										Majority	
		30													40														
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	E	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415VK
28		N	S	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK1
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK2
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK3
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK4
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK5
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	E	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK6
28		N	V	G	T	Y	V	S	W	Y	Q	Q	K	P	T	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK7
28		N	S	G	T	Y	V	S	W	Y	Q	Q	K	P	E	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415DIVK8
		F T G V P D R F S G S G S G T D F I L T I S S V Q A E																										Majority	
		60													70														
55		F	T	G	V	P	D	R	F	S	G	S	G	S	A	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415VK
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	A	S	S	V	Q	A	E	J415DIVK1
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	A	S	S	V	Q	A	E	J415DIVK2
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	A	S	S	V	Q	A	E	J415DIVK3
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415DIVK4
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415DIVK5
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415DIVK6
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415DIVK7
55		F	T	G	V	P	D	R	F	S	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	J415DIVK8
		D L V D Y Y C G Q S Y T F P Y T F G G G T K L E M K																										Majority	
		90													100														
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415VK	
82		D	P	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK1	
82		D	P	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK2	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK3	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK4	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK5	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK6	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK7	
82		D	L	V	D	Y	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K	J415DIVK8	

FIG. 6

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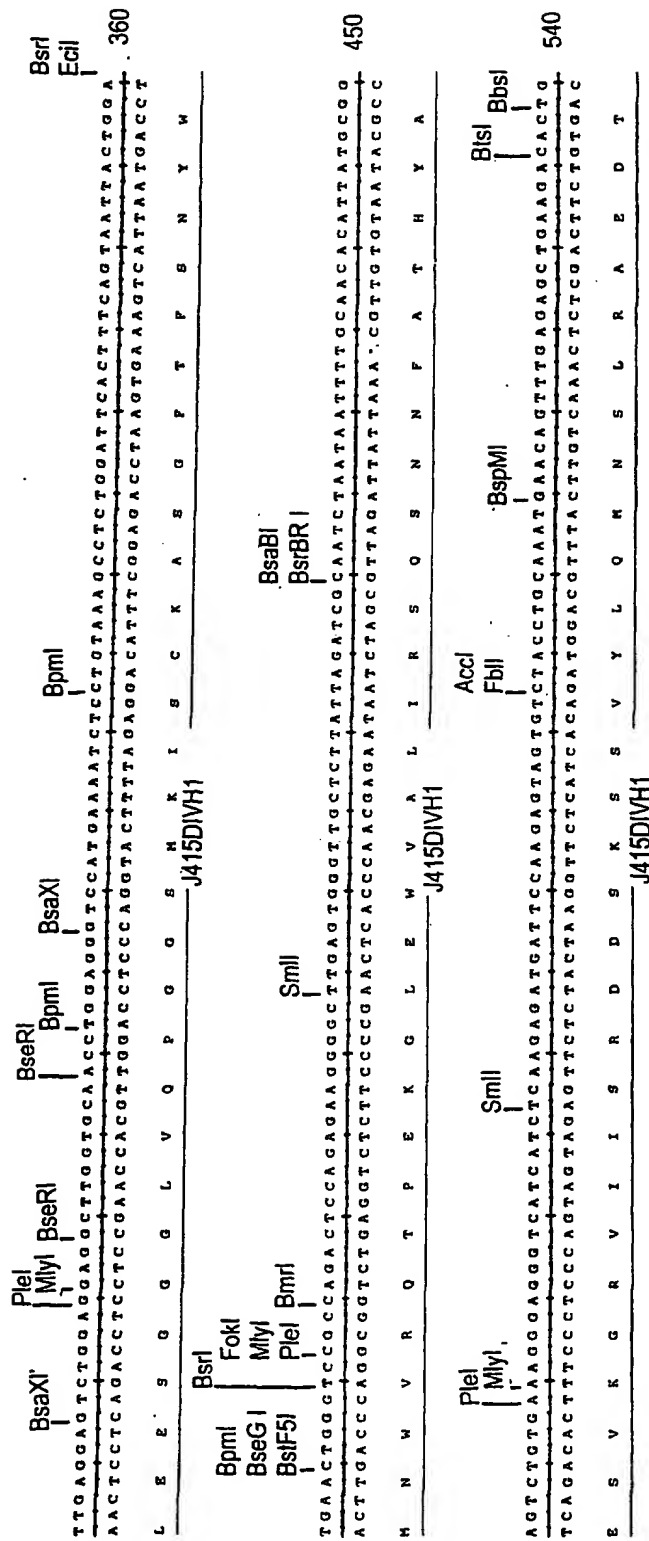


FIG. 7A-2

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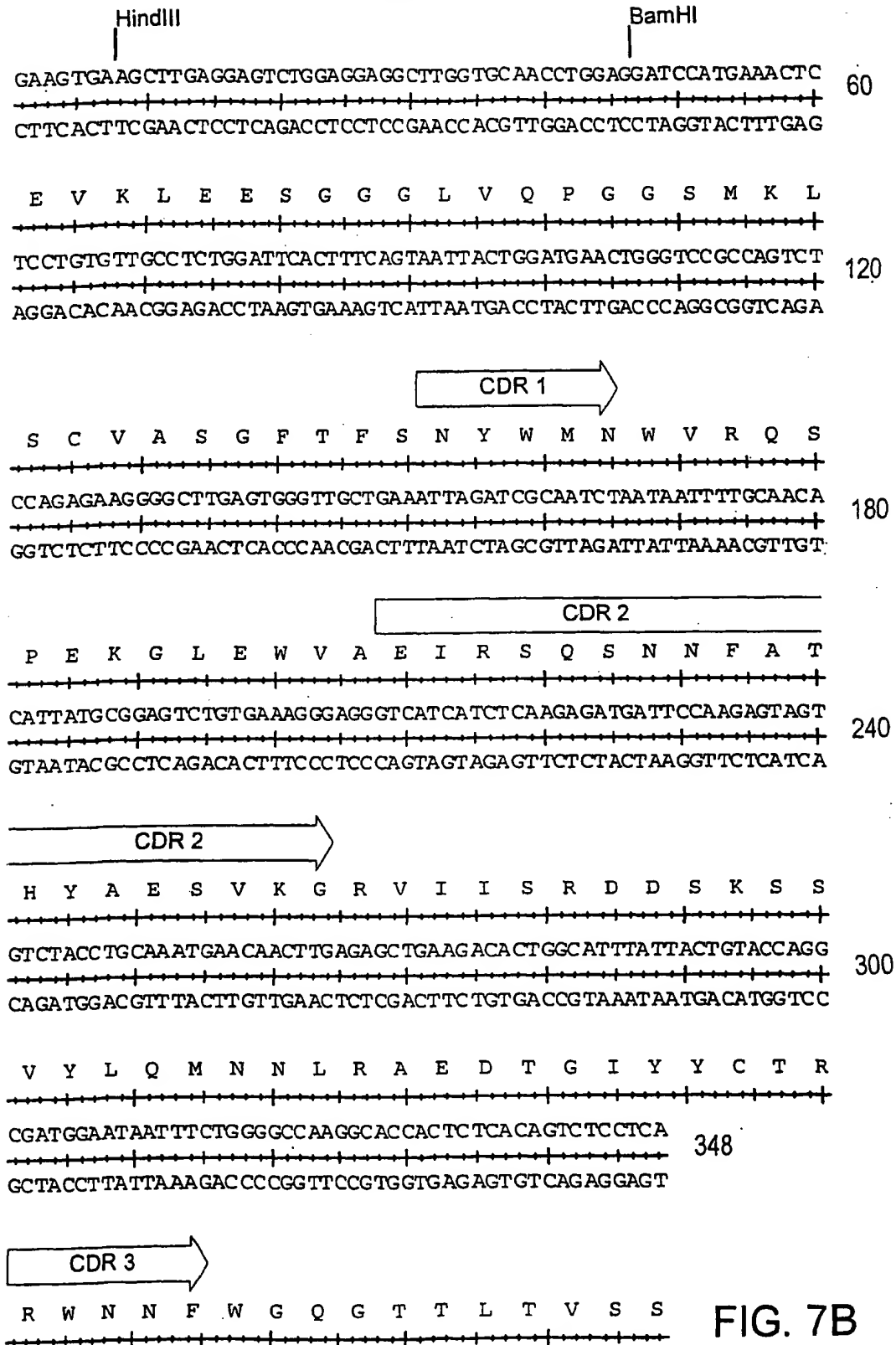
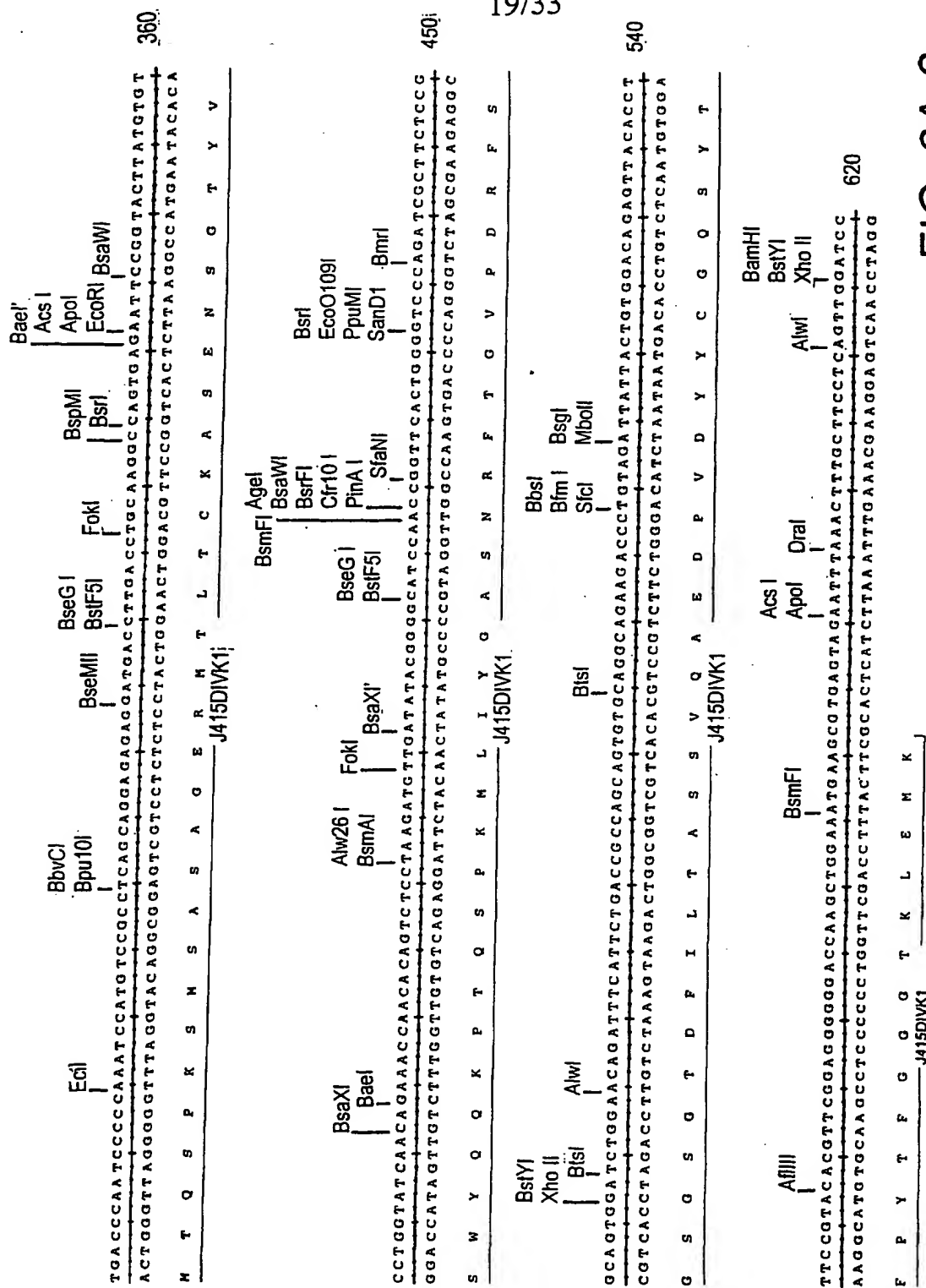


FIG. 7B

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	E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S	Majority
	10 20 30	
1	E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S	J415vh
1	E V K L E E S G G G L V Q P G G S M K L S C V A S G F T F S	MUVHIIIIO
	N Y W M N W V R Q S P E K G L E W V A E I R L Q S D N F A T	Majority
	40 50 60	
31	N Y W M N W V R Q S P E K G L E W V A E I R S Q S N N F A T	J415vh
31	N Y W M N W V R Q S P E K G L E W V A E I R L K S D N Y A T	MUVHIIIIO
	H Y A E S V K G R V I I S R D D S K S S V Y L Q M N N L R A	Majority
	70 80 90	
61	H Y A E S V K G R V I I S R D D S K S S V Y L Q M N N L R A	J415vh
61	H Y A E S V K G R F T I S R D D S K S S V Y L Q M N N L R A	MUVHIIIIO
	E D T G I Y Y C T T G G Y G G R R S W N A F W G Q G T L V T	Majority
	100 110 120	
91	E D T G I Y Y C T - - - - - R R W N N F W G Q G T T L T	J415vh
91	E D T G I Y Y C T T G G Y G G R R S W F A Y W G Q G T L V T	MUVHIIIIO
	<u>V S S</u>	Majority
114	<u>V S S</u>	J415vh
121	<u>V S S</u>	MUVHIIIIO

FIG. 7C



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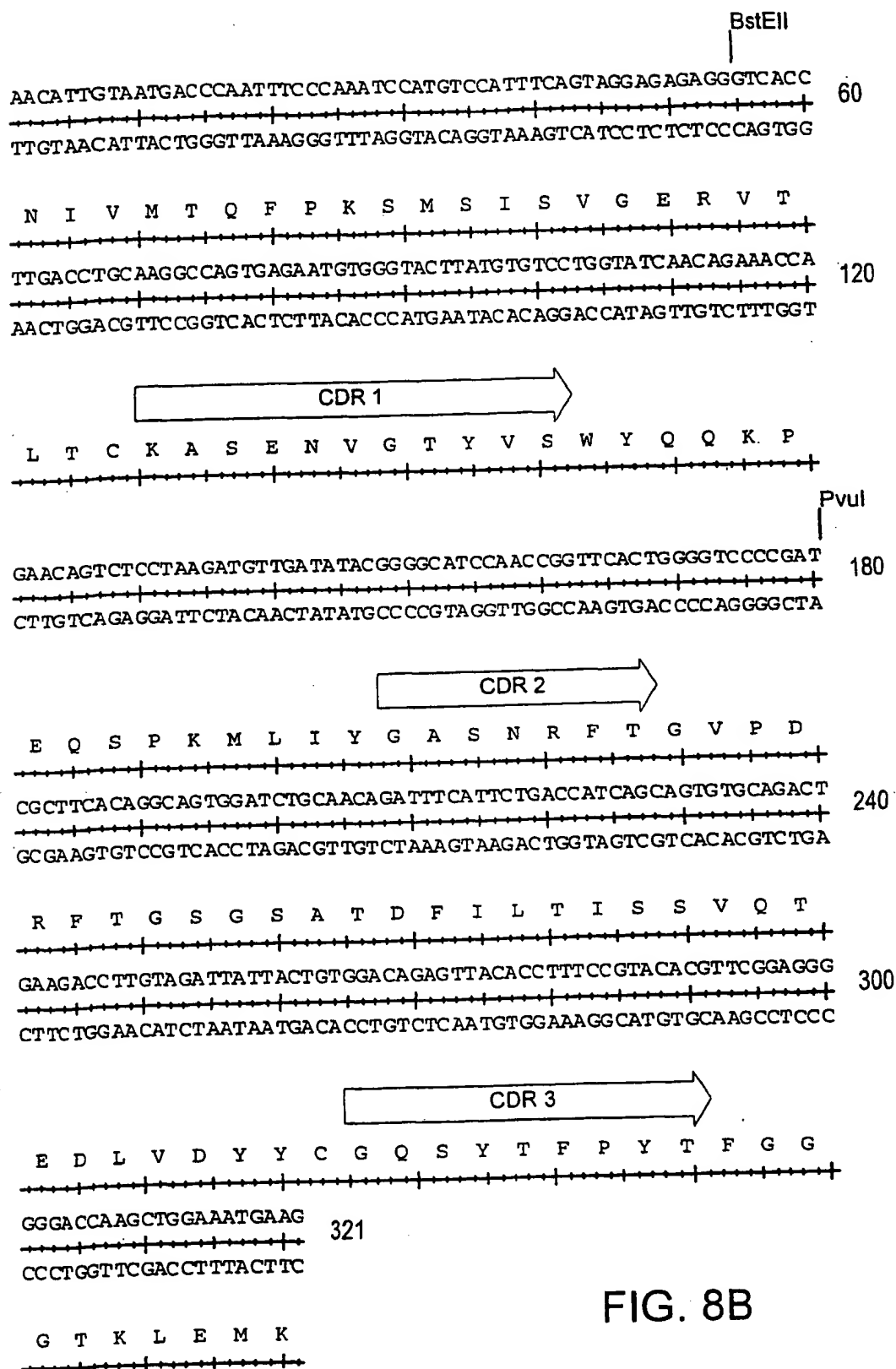


FIG. 8B

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	D	I	V	M	T	Q	S	P	S	S	L	A	V	S	A	G	E	K	V	T	L	S	C	K	A	S	E	S	L	L	Majori
										10											20									30	
1	N	I	V	M	T	Q	F	P	K	S	M	S	I	S	V	G	E	R	V	T	L	T	C	K	A	S	E	-	-	-	J415vk
1	D	I	V	M	T	Q	S	P	S	S	L	A	V	S	A	G	E	K	V	T	M	S	C	K	S	Q	S	L	L	L	Muvk1
	N	V	G	N	Q	K	T	Y	V	A	W	Y	Q	Q	K	P	G	Q	S	P	K	L	I	Y	G	A	S	T	R	Majori	
										40											50									60	
28	N	V	G	-	-	-	T	Y	V	S	W	Y	Q	Q	K	P	E	Q	S	P	K	M	L	I	Y	G	A	S	N	R	J415vk
31	N	S	G	N	Q	K	N	Y	L	A	W	Y	Q	Q	K	P	G	Q	S	P	K	L	I	Y	W	A	S	T	R	Muvk1	
	E	S	G	V	P	D	R	F	T	G	S	G	S	G	T	D	F	I	L	T	I	S	S	V	Q	A	E	D	L	A	Majori
										70											80									90	
55	F	T	G	V	P	D	R	F	T	G	S	G	S	A	T	D	F	I	L	T	I	S	S	V	Q	T	E	D	L	V	J415vk
61	E	S	G	V	P	D	R	F	T	G	S	G	S	G	T	D	F	T	I	S	S	V	Q	A	E	D	L	A	Muvk1		
	V	Y	C	G	N	S	Y	S	F	P	L	T	F	G	G	G	T	K	L	E	L	K								Majori	
										100											110										
85	D	Y	C	G	Q	S	Y	T	F	P	Y	T	F	G	G	G	T	K	L	E	M	K								J415vk	
91	V	Y	C	Q	N	D	Y	S	Y	P	L	T	F	G	A	G	T	K	L	E	L	K								Muvk1	

FIG. 8C

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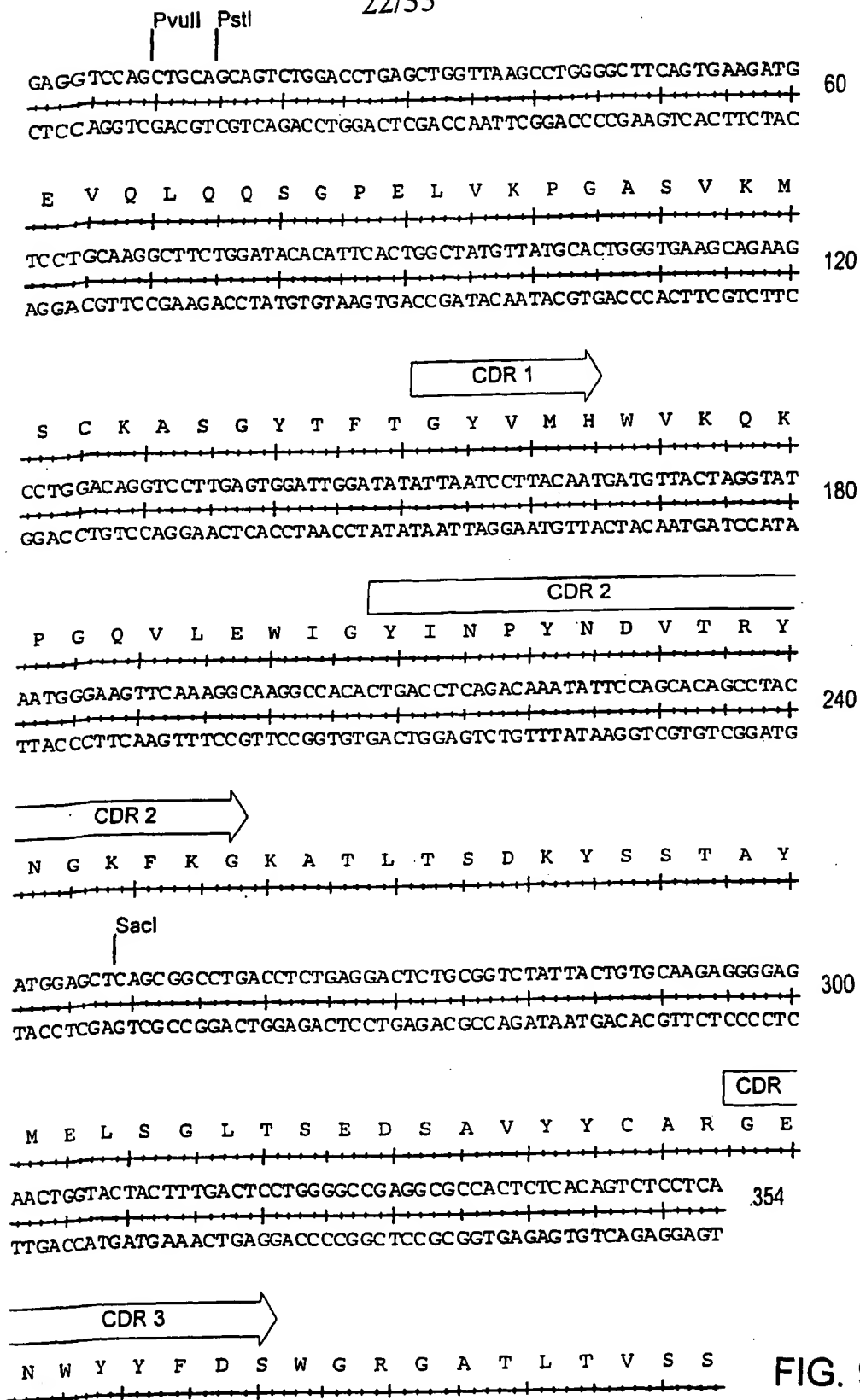


FIG. 9A

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1	EVQLQQSGPELVKPGASVKISCKASGYTF	10	20	30	Majori
11	EVQLQQSGPELVKPGASVKISCKASGYTF				j533vh
11	EVQLQQSGPELVKPGASVKISCKASGYTF				Muvhii
	GYVMNNWVKQSPGGVLEWIGDINPGNGGTS	40	50	60	Majori
31	GYVMH-WVKQKPGGVLEWIGYINPYNDVTR				j533vh
31	DY[YN]NWKQ[S]PG[K]SLEWIG[D]INP[G]N[G]G[T]S				Muvhii
	YNGKFKGKATLTVDKSSSTAYMELSLTSE	70	80	90	Majori
60	YNGKFKGKATLTSDKYSSSTAYMELSLTSE				j533vh
61	YN[Q]KFKGKATLT[V]DK[S]SSTAYM[Q]LS[SLTSE				Muvhii
	DSAVYYCARGENS SYMAYYAFDSWQGQAT	100	110	120	Majori
90	DSAVYYCARGEN- - - - -WYYFDSWGRGAT				j533vh
91	DSAVYYCARGY[YS]SSSYMA[Y][A]FD[Y]WG[Q]G[T]T				Muvhii
	VTVSS				Majori
114	LTVSS				j533vh
21	VTVSS				Muvhii

FIG. 9B

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GACATTGTGCTGACCCAATCTCAGCTCTTTGGCTGTGTCTCTAGGACAGAGGGCCACC
CTGTAACACGACTGGGTAGAGGTGAAGAAACCGACACAGAGATCCTGTCTCCCGGTGG

D I V L T Q S P A S L A V S L G Q R A T

PstI KpnI
ATATCCTGCAGAGCCAGTGAAAGTATTGATAGTTATGACAATACTTTATGCACTGGTAC 120
TATAGGACGTCTCGGTCACTTTCATAACTATCAATACGTTATGAAAATACGTGACCATG

CDR 1
I S C R A S E S I D S Y D N T F M H W Y
CAGCAGAAACGAGGACAGCCACCAACCTCCTCATCTTTCGTGCATCCATCCTAGAATCT 180
GTCGTCTTTGGTCTGTCTCGGTGGGTGGAGGAGTAGAAAGCACGTAGGTAGGATCTTAGA

CDR 2
Q Q K P G Q P P N L L I F R A S I L E S
BamHI
GGGATCCCTGCCAGGTTCACTGGCAGTGGGTCGGGACAGACTTCACCCTCACCATTTAT 240
CCCTAGGGACGGTCCAAGTCACCGTCAACAGACCTGTCTGAAGTGGGAGTGGTAAATA

G I P A R F S G S G S G T D F T L T I Y
BamHI
CCTGTGGAGGCTGATGATGTTGCAACCTATTACTGTCACCAAAGTATTGAGGATCCGTAC 300
GGACACCTCCGACTACTACAACGTGGATAATGACAGTGTTTCATAACTCCTAGGCATG

CDR 3
P V E A D D V A T Y Y C H Q S I E D P Y
ACGTTTCGGAGGGGGGACCAAGCTGGAAATAAAA 333
TGCAAGCCTCCCCCTGGTTCGACCTTTATTTT

T F G G G T K L E I K

FIG. 10A

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	D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S V D	Majori
	10 20 30	
21	D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S I D	j533vk
1	D I V L T Q S P A S L A V S L G Q R A T I S C R A S E S V D	Muvk3
	S Y G N S F M H W Y Q Q K P G Q P P N L L I F A A S I L E S	Majori
	40 50 60	
51	S Y D N T F M H W Y Q Q K P G Q P P N L L I F R A S I L E S	j533vk
31	S Y G N S F M H W Y Q Q K P G Q P P K L L I Y A A S N L E S	Muvk3
	G V P A R F S G S G T D F T L T I H P V E A D D A A T Y	Majori
	70 80 90	
81	G I P A R F S G S G T D F T L T I Y P V E A D D V A T Y	j533vk
61	G V P A R F S G S G T D F T L N I H P V E D D A A T Y	Muvk3
	Y C Q Q S I E D P P Y T F G G G T K L E I K	Majori
	100 110	
111	Y C H Q S I E D P P Y T F G G G T K L E I K	j533vk
91	Y C Q Q S N E D P P W T F G G G T K L E I K	Muvk3

FIG. 10B

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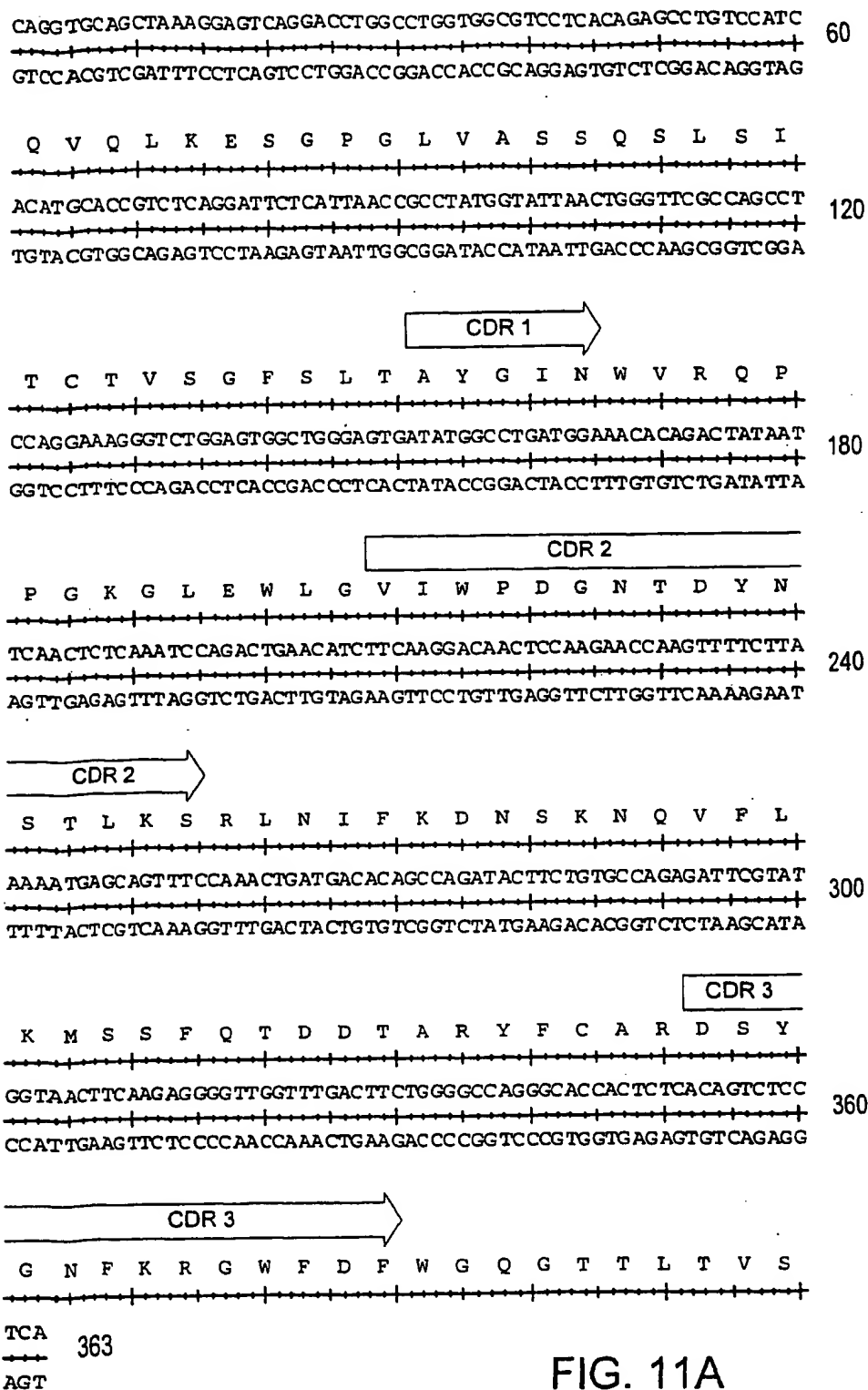


FIG. 11A

1	QVQLKESGPGGLVASSQSLSITCTVSGFSLT	10	20	30	Majori
11	QVQLKESGPGGLVASSQSLSITCTVSGFSLT				E99vh
11	QVQLKESGPGGLV[P]SQSLSITCTVSGFSLT				Muvhib
	AYGVNVSVVRQPPGKGLEWLGV I WAGGST D				Majori
		40	50	60	
31	AYGIN[-]WVRQPPGKGLEWLGV I WPDGNT D				E99vh
31	[S]YG[V]HVS WVRQPPGKGLEWLGV I W[A]G[S]T[N]				Muvhib
	YNSALKSRLSISKDNSK SQVFLKMS SLQT D				Majori
		70	80	90	
59	YNSTLKSRRLNIFKDNSKNQVFLKMS SFQTT D				E99vh
61	YNS[A]L[M]SRRL[S]ISKDNSK[S]QVFLK[M]N[S]L]QTT D				Muvhib
	DTARYFCARDSGGNFKSGYFAMDFWQQGTS				Majori
		100	110	120	
89	DTARYFCARDSYGNFKRGWF[-]DFWQQGTT				E99vh
91	DTA[M]Y[Y]CARD[R]GRYYYS[G]YYAM[D]YWGQGT[S]				Muvhib
	VT VSS				Majori
117	LT VSS				E99vh
121	[V]TVSS				Muvhib

FIG. 11B

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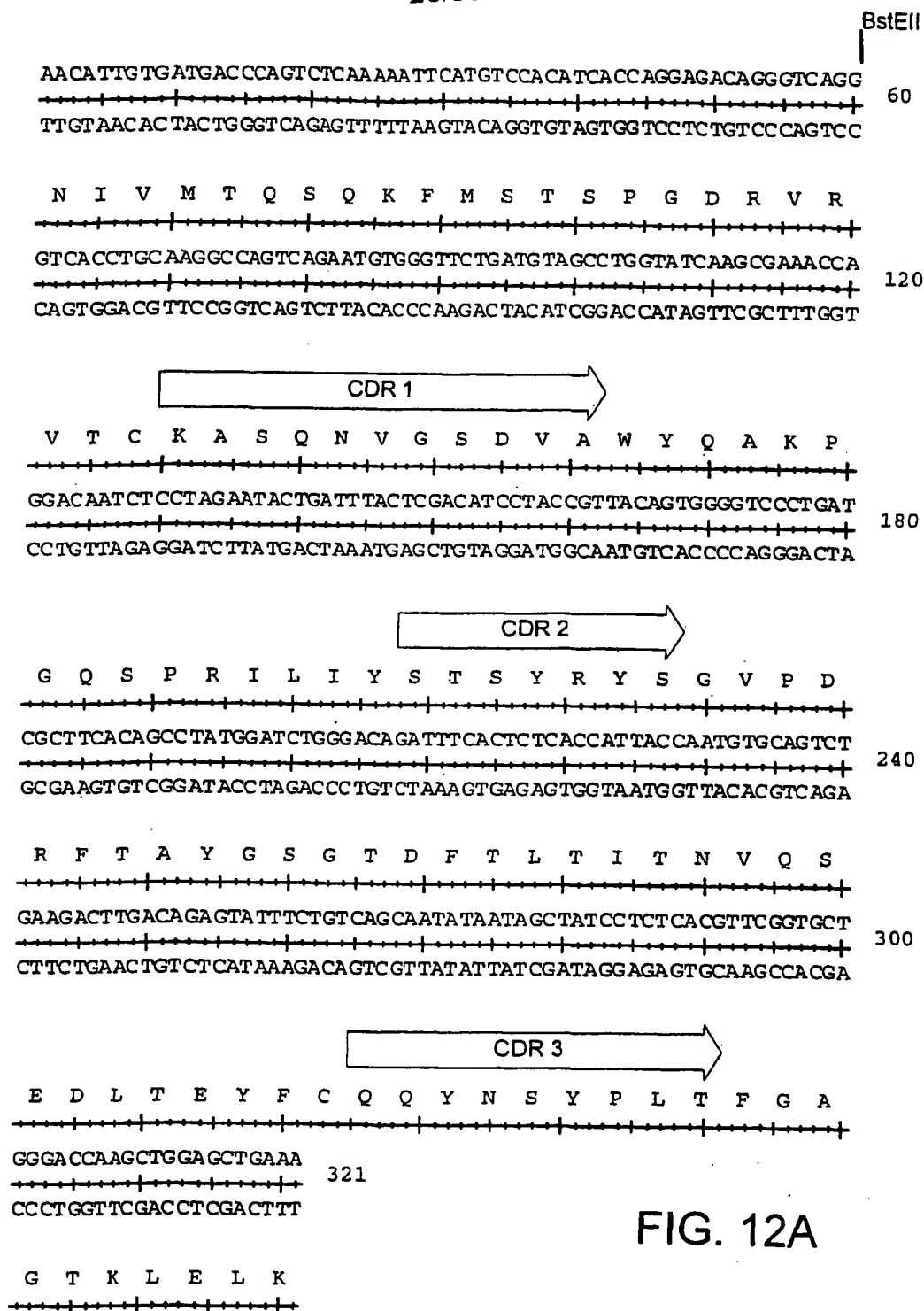


FIG. 12A

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1	D	I	V	M	T	Q	S	Q	S	S	L	A	V	S	A	G	D	K	V	T	V	S	C	K	A	S	Q	S	L	L	Majori

FIG. 12B

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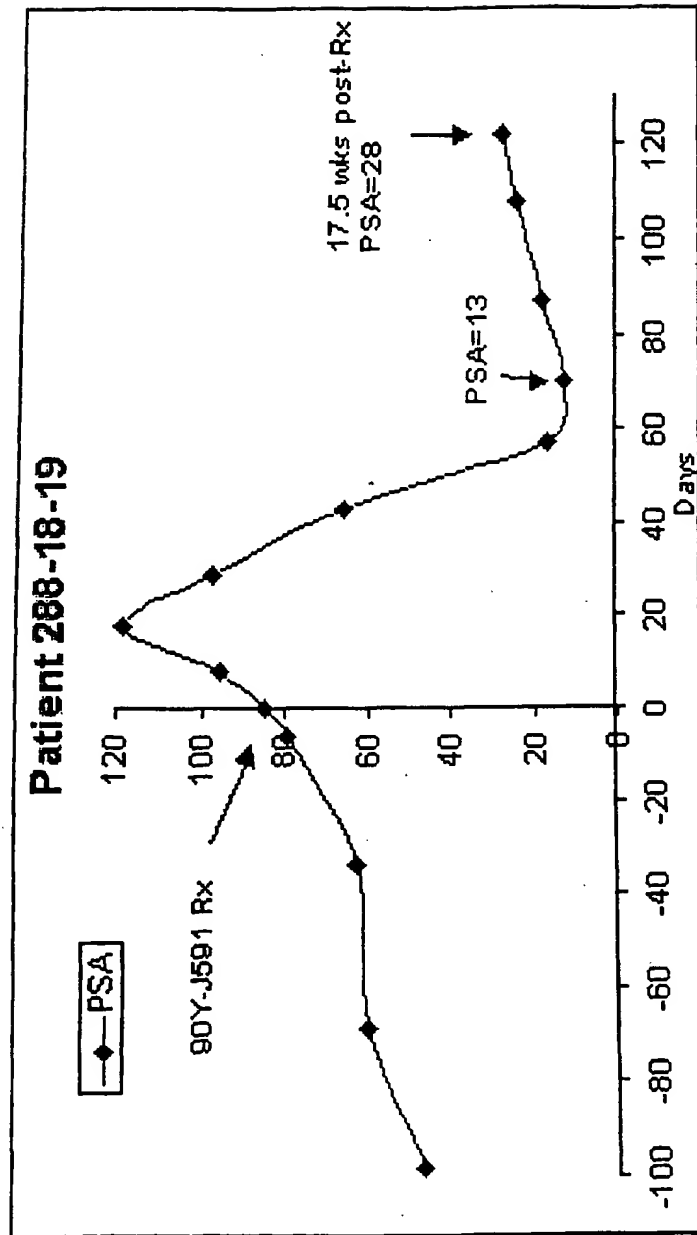


FIG. 13A

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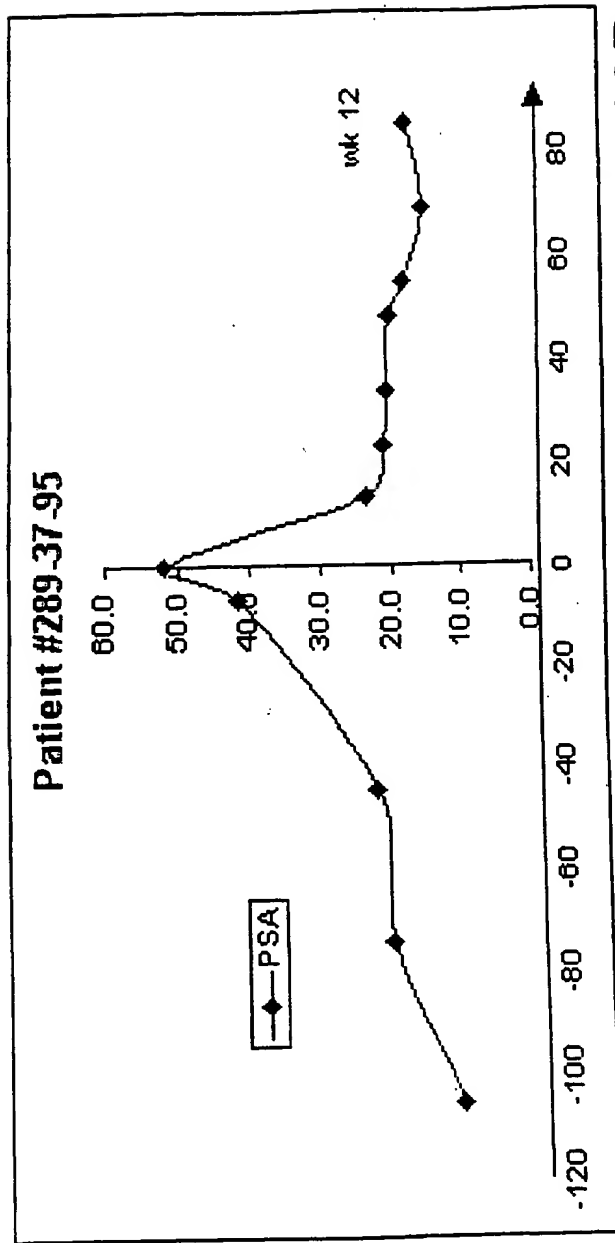


FIG. 13B

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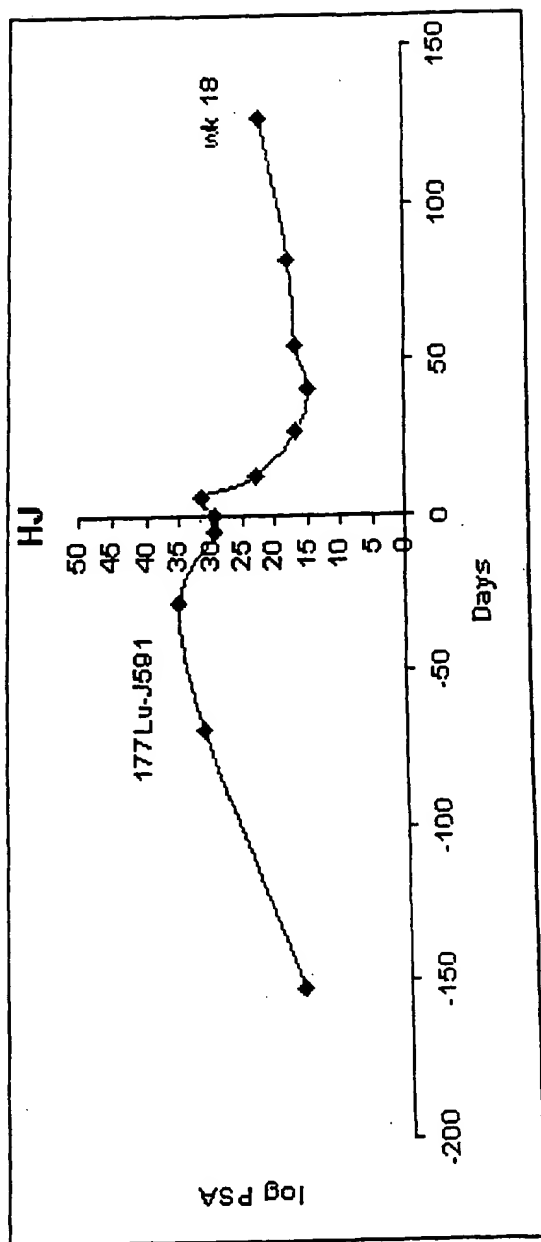
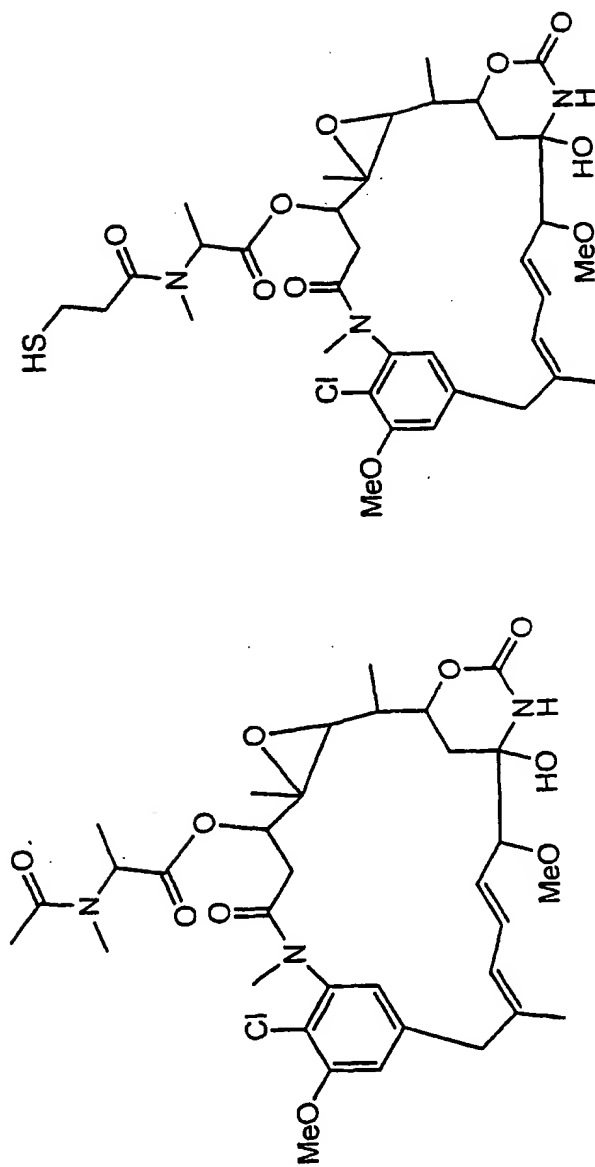


FIG. 14

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$C_{35}H_{48}ClN_3O_{10}S$
Exact Mass: 737.27
Mol. Wt.: 738.29
DMI

$C_{34}H_{46}ClN_3O_{10}$
Exact Mass: 691.29
Mol. Wt.: 692.20
Maytansine

FIG. 15

SEQUENCE LISTING

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<120> MODIFIED ANTIBODIES TO PROSTATE-SPECIFIC
MEMBRANE ANTIGEN AND USES THEREOF

<130> 10448-163W01

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<151> 2001-09-20

<150> 60/362,810

<151> 2002-03-08

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<210> 2

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<212> PRT

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1 5 10 15
Asp

<210> 3

<211> 6

<212> PRT

<213> Mus musculus

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1 5

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 1 5

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 1 5

<210> 7
 <211> 82
 <212> PRT
 <213> Mus musculus

<400> 7
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Thr
 1 5 10 15
 Ser Val Arg Ile Ser Cys Lys Thr Ser Trp Val Lys Gln Ser His Gly
 20 25 30
 Lys Ser Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
 35 40 45
 Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser
 50 55 60
 Ala Val Tyr Tyr Cys Ala Ala Trp Gly Gln Gly Thr Thr Leu Thr Val
 65 70 75 80
 Ser Ser

<210> 8
 <211> 80
 <212> PRT
 <213> Mus musculus

<400> 8
 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
 1 5 10 15
 Asp Arg Val Ser Ile Ile Cys Trp Tyr Gln Gln Lys Pro Gly Gln Ser
 20 25 30
 Pro Lys Leu Leu Ile Tyr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
 35 40 45
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Glu Asp
 50 55 60
 Leu Ala Asp Tyr Phe Cys Phe Gly Ala Gly Thr Met Leu Asp Leu Lys
 65 70 75 80

<210> 9
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J591


```
<210> 14
<211> 15
```

<212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

<400> 14
 Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile Tyr
 1 5 10 15

<210> 15
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

<400> 15
 Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15
 Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Asp Tyr Tyr Cys
 20 25 30

<210> 16
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

<400> 16
 Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
 1 5 10

<210> 17
 <211> 82
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J591

<400> 17
 Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Thr Val Lys Ile Ser Cys Lys Thr Ser Trp Val Lys Gln Ala Pro Gly
 20 25 30
 Lys Gly Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Val Asp Lys Ser
 35 40 45
 Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
 50 55 60
 Ala Val Tyr Tyr Cys Ala Ala Trp Gly Gln Gly Thr Leu Leu Thr Val
 65 70 75 80
 Ser Ser

<210> 18
 <211> 80
 <212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J591

<400> 18

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr Ser Val Gly
 1           5           10           15
Asp Arg Val Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Gly Pro Ser
 20           25           30
Pro Lys Leu Leu Ile Tyr Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly
 35           40           45
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 50           55           60
Phe Ala Asp Tyr Tyr Cys Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
 65           70           75           80

```

<210> 19

<211> 115

<212> PRT

<213> Mus musculus

<400> 19

```

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Thr
 1           5           10           15
Ser Val Arg Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
 20           25           30
Thr Ile His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
 35           40           45
Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe
 50           55           60
Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65           70           75           80
Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85           90           95
Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
100           105           110
Val Ser Ser
115

```

<210> 20

<211> 107

<212> PRT

<213> Mus musculus

<400> 20

```

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
 1           5           10           15
Asp Arg Val Ser Ile Ile Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
 20           25           30
Val Asp Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
 35           40           45
Tyr Trp Ala Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly
 50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
 65           70           75           80
Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
 85           90           95
Thr Phe Gly Ala Gly Thr Met Leu Asp Leu Lys
100           105

```

<210> 21
 <211> 115
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J591

<400> 21
 Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Thr Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr
 20 25 30
 Thr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe
 50 55 60
 Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asp Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr Leu Leu Thr
 100 105 110
 Val Ser Ser
 115

<210> 22
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

<400> 22
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Leu Thr Cys Lys Ala Ser Gln Asp Val Gly Thr Ala
 20 25 30
 Val Asp Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile
 35 40 45
 Tyr Trp Ala Ser Thr Arg His Thr Gly Ile Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Asp Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Leu
 85 90 95
 Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
 100 105

<210> 23
 <211> 807
 <212> DNA
 <213> Artificial Sequence

<220>
 <221> CDS
 <222> (122)...(166)

<221> CDS
 <222> (249)...(605)

<223> deimmunized heavy chain J591

<400> 23

aagcttatga atatgcaa at cctctgaatc tacatggtaa atatagggtt gtctatacca 60
 caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
 c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca 166
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
 1 5 10 15

ggtaaggggc tcacagtagc aggcttgagg tctggacata tatatgggtg acaatgacat 226
 ccactttgcc tttctctcca ca ggt gtc cac tcc gag gtc caa ctg gta cag 278
 Gly Val His Ser Glu Val Gln Leu Val Gln
 20 25

tct gga cct gaa gtg aag aag cct ggg gct aca gtg aag ata tcc tgc 326
 Ser Gly Pro Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys
 30 35 40

aag act tct gga tac aca ttc act gaa tat acc ata cac tgg gtg aag 374
 Lys Thr Ser Gly Tyr Thr Phe Thr Glu Tyr Thr Ile His Trp Val Lys
 45 50 55

cag gcc cct gga aag ggc ctt gag tgg att gga aac atc aat cct aac 422
 Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Asn Ile Asn Pro Asn
 60 65 70

aat ggt ggt acc acc tac aat cag aag ttc gag gac aag gcc aca cta 470
 Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe Glu Asp Lys Ala Thr Leu
 75 80 85

act gta gac aag tcc acc gat aca gcc tac atg gag ctc agc agc cta 518
 Thr Val Asp Lys Ser Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser Leu
 90 95 100 105

aga tct gag gat act gca gtc tat tat tgt gca gct ggt tgg aac ttt 566
 Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Gly Trp Asn Phe
 110 115 120

gac tac tgg ggc caa ggg acc ctg ctc acc gtc tcc tca ggtgagtcct 615
 Asp Tyr Trp Gly Gln Gly Thr Leu Thr Val Ser Ser
 125 130

tacaacctct ctcttctatt cagcttaa at agattttact gcatttggtg ggggggaaat 675
 gtgtgtatct gaatttcagg tcatgaagga ctaggacac cttgggagtc agaaagggtc 735
 attgggagcc cgggctgatg cagacagaca tcctcagctc ccagacttca tggccagaga 795
 tttataggat cc 807

<210> 24

<211> 807

<212> DNA

<213> Artificial Sequence

<220>

<223> deimmunized heavy chain J591

<400> 24

ggatcctata aatctctggc catgaagtct gggagctgag gatgtctgtc tgcacagcc 60
 cgggctccca atgaccttt ctgactccca aggtgtccct agtccttcat gacctgaaat 120
 tcagatacac acatttcccc cccaacaaat gcagtaaaat ctatttaagc tgaatagaag 180
 agagagggtg taaggactca cctgaggaga cggtgagcag ggtcccttgg cccagtagt 240

```

caaagttcca accagctgca caataataga ctgcagtatc ctcagatctt aggctgctga 300
gctccatgta ggctgtatcg gtggacttgt ctacagttag tgtggccttg tcctcgaact 360
tctgattgta ggtggtacca ccattgttag gattgatgtt tccaatccac tcaaggccct 420
ttccaggggc ctgcttcacc cagtgtatgg tatattcagt gaatgtgtat ccagaagtct 480
tgcaggatat cttcactgta gcccaggct tcttcacttc aggtccagac tgtaccagtt 540
ggacctcgga gtggacacct gtggagagaa aggcaaagtg gatgtcattg tcacccatat 600
atatgtccag acctcaagcc tgctactgtg agccccttac ctgtagctgt tgctaccaag 660
aagaggatga tacagctcca tcccatggtg aggtcctgtg tgctcagtaa ctgtagagag 720
aactgtgatc tcatgttttt ctgtttgtgg tatagacaaa cctatatatta ccatgtagat 780
tcagaggatt tgcattattca taagctt 807

```

<210> 25

<211> 620

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (122) ... (166)

<221> CDS

<222> (249) ... (581)

<223> deimmunized light chain J591

<400> 25

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aagcttatga atatgcaa at cctctgaatc tacatggtaa atatagggtt gtctatacca 60
caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca 166
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr
1 5 10 15

```

```

ggtaaggggc tcacagtagc aggcttgagg tctggacata tatatgggtg acaatgacat 226
ccactttgcc tttctctcca ca ggt gtc cac tcc gac atc cag atg acc cag 278
Gly Val His Ser Asp Ile Gln Met Thr Gln
20 25

```

```

tct ccc tca tcc ctg tcc aca tca gta gga gac agg gtc acc ctc acc 326
Ser Pro Ser Ser Leu Ser Thr Ser Val Gly Asp Arg Val Thr Leu Thr
30 35 40

```

```

tgt aag gcc agt caa gat gtg ggt act gct gta gac tgg tat caa cag 374
Cys Lys Ala Ser Gln Asp Val Gly Thr Ala Val Asp Trp Tyr Gln Gln
45 50 55

```

```

aaa cca gga cca tct cct aaa cta ctg att tat tgg gca tcc act cgg 422
Lys Pro Gly Pro Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
60 65 70

```

```

cac act gga atc cct agt cgc ttc tca ggc agt gga tct ggg aca gac 470
His Thr Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
75 80 85

```

```

ttc act ctc acc att tct agt ctt cag cct gaa gac ttt gca gat tat 518
Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Asp Tyr
90 95 100 105

```

```

tac tgt cag caa tat aac agc tat cct ctc acg ttc ggt cct ggg acc 566
Tyr Cys Gln Gln Tyr Asn Ser Tyr Pro Leu Thr Phe Gly Pro Gly Thr
110 115 120

```

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aag gtg gac atc aaa cgtgagtaga atttaaactt tgcttcctca gttggatcc 620
 Lys Val Asp Ile Lys
 .125

<210> 26
 <211> 620
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

<400> 26
 ggatccaact gaggaagcaa agtttaaatt ctactcacgt ttgatgtcca ccttggtccc 60
 aggaccgaac gtgagaggat agctgttata ttgctgacag taataatctg caaagtcttc 120
 aggctgaaga ctagaaatgg tgagagtga gtcgtgccca gatccactgc ctgagaagcg 180
 actagggatt ccagtgtgcc gagtggatgc ccaataaatc agtagtttag gagatgggtcc 240
 tgggtttctgt tgataccagt ctacagcagt acccacatct tgactggcct tacaggtgag 300
 ggtgaccctg tctcctactg atgtggacag ggatgaggga gactgggtca tctggatgtc 360
 ggagtggaca cctgtggaga gaaaggcaaa gtggatgtca ttgtcaccca tatatatgtc 420
 cagacctcaa gcctgctact gtgagccctt tacctgtagc tgttgctacc aagaagagga 480
 tgatacagct ccatcccatg gtgaggtcct gtgtgctcag taactgtaga gagaactgtg 540
 atctcatgtt tttctgtttg tggtagatag aaacctatat ttaccatgta gattcagagg 600
 atttgcataat tcataagctt 620

<210> 27
 <211> 134
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J591

<400> 27
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15
 Val His Ser Glu Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
 20 25 30
 Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe
 35 40 45
 Thr Glu Tyr Thr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
 50 55 60
 Glu Trp Ile Gly Asn Ile Asn Pro Asn Asn Gly Gly Thr Thr Tyr Asn
 65 70 75 80
 Gln Lys Phe Glu Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Thr Asp
 85 90 95
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Ala Gly Trp Asn Phe Asp Tyr Trp Gly Gln Gly Thr
 115 120 125
 Leu Leu Thr Val Ser Ser
 130

<210> 28
 <211> 126
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J591

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<400> 28

```

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1           5           10           15
Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Thr
          20           25           30
Ser Val Gly Asp Arg Val Thr Leu Thr Cys Lys Ala Ser Gln Asp Val
      35           40           45
Gly Thr Ala Val Asp Trp Tyr Gln Gln Lys Pro Gly Pro Ser Pro Lys
      50           55           60
Leu Leu Ile Tyr Cys Ala Ser Thr Arg His Thr Gly Ile Pro Ser Arg
65           70           75           80
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser
          85           90           95
Leu Gln Pro Glu Asp Phe Ala Asp Tyr Tyr Cys Gln Gln Tyr Asn Ser
          100          105          110
Tyr Pro Leu Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
      115           120           125

```

<210> 29

<211> 10

<212> PRT

<213> Mus musculus

<400> 29

```

Gly Phe Thr Phe Ser Asn Tyr Trp Met Asn
 1           5           10

```

<210> 30

<211> 19

<212> PRT

<213> Mus musculus

<400> 30

```

Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu Ser
 1           5           10           15
Val Lys Gly

```

<210> 31

<211> 5

<212> PRT

<213> Mus musculus

<400> 31

```

Arg Trp Asn Asn Phe
 1           5

```

<210> 32

<211> 11

<212> PRT

<213> Mus musculus

<400> 32

```

Lys Ala Ser Glu Asn Val Gly Thr Tyr Val Ser
 1           5           10

```

<210> 33

<211> 7

<212> PRT

<213> Mus musculus

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<400> 33
 Gly Ala Ser Asn Arg Phe Thr
 1 5

<210> 34
 <211> 9
 <212> PRT
 <213> Mus musculus

<400> 34
 Gly Gln Ser Tyr Thr Phe Pro Tyr Thr
 1 5

<210> 35
 <211> 82
 <212> PRT
 <213> Mus musculus

<400> 35
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Met Lys Leu Ser Cys Val Ala Ser Trp Val Arg Gln Ser Pro Glu
 20 25 30
 Lys Gly Leu Glu Trp Val Ala Arg Val Ile Ile Ser Arg Asp Asp Ser
 35 40 45
 Lys Ser Ser Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr
 50 55 60
 Gly Ile Tyr Tyr Cys Thr Arg Trp Gly Gln Gly Thr Thr Leu Thr Val
 65 70 75 80
 Ser Ser

<210> 36
 <211> 80
 <212> PRT
 <213> Mus musculus

<400> 36
 Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ile Ser Val Gly
 1 5 10 15
 Glu Arg Val Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Glu Gln Ser
 20 25 30
 Pro Lys Met Leu Ile Tyr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
 35 40 45
 Ser Ala Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Thr Glu Asp
 50 55 60
 Leu Val Asp Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 65 70 75 80

<210> 37
 <211> 25
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-4

<400> 37
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

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Ser Met Lys Ile Ser Cys Val Ala Ser
20 25

<210> 38
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 38
Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val Ala
1 5 10

<210> 39
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 39
Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser Val Tyr Leu Gln
1 5 10 15
Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg
20 25 30

<210> 40
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized heavy chain J415-4

<400> 40
Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
1 5 10

<210> 41
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-5

<400> 41
Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys
20

<210> 42
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 42

Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile Tyr
 1 5 10 15

<210> 43

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 43

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ile
 1 5 10 15
 Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Val Asp Tyr Tyr Cys
 20 25 30

<210> 44

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 44

Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 1 5 10

<210> 45

<211> 82

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized heavy chain J415-4

<400> 45

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Met Lys Ile Ser Cys Val Ala Ser Trp Val Arg Gln Ser Pro Glu
 20 25 30
 Lys Gly Leu Glu Trp Val Ala Arg Val Ile Ile Ser Arg Asp Asp Ser
 35 40 45
 Lys Ser Ser Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
 50 55 60
 Ala Val Tyr Tyr Cys Thr Arg Trp Gly Gln Gly Thr Thr Val Thr Val
 65 70 75 80
 Ser Ser

<210> 46

<211> 80

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 46

```

Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1           5           10           15
Glu Arg Met Thr Leu Thr Cys Trp Tyr Gln Gln Lys Pro Thr Gln Ser
          20          25          30
Pro Lys Met Leu Ile Tyr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
          35          40          45
Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
          50          55          60
Leu Val Asp Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
65          70          75          80

```

<210> 47

<211> 116

<212> PRT

<213> Mus musculus

<400> 47

```

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1           5           10           15
Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
          20          25          30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
          35          40          45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
          50          55          60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
65          70          75          80
Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
          85          90          95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Leu
          100          105          110
Thr Val Ser Ser
          115

```

<210> 48

<211> 107

<212> PRT

<213> Mus musculus

<400> 48

```

Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ile Ser Val Gly
 1           5           10           15
Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
          20          25          30
Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
          35          40          45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Thr Gly
          50          55          60
Ser Gly Ser Ala Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Thr
65          70          75          80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
          85          90          95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
          100          105

```

<210> 49

<211> 116

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized heavy chain J415-4

<400> 49

```

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1           5           10           15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20           25           30
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
 35           40           45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
 50           55           60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
 65           70           75           80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
 85           90           95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
 100          105          110
Thr Val Ser Ser
 115

```

<210> 50

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-5

<400> 50

```

Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1           5           10           15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
 20           25           30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
 35           40           45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
 50           55           60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
 65           70           75           80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
 85           90           95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 100          105

```

<210> 51

<211> 348

<212> DNA

<213> Artificial Sequence

<220>

<223> deimmunized heavy chain J415-4

<400> 51

```

gaagtgaac ttgaggagtc tggaggaggc ttggtgcaac ctggagggtc catgaaaatc      60
tctgtgttg cctctggatt cactttcagt aattactgga tgaactgggt ccgccagtct      120
ccagagaagg ggcttgagtg ggttgctgaa attagatcgc aatctaataa ttttgcaaca      180
cattatgcgg agtctgtgaa agggagggtc atcatctcaa gagatgattc caagagtagt      240
gtctacctgc aaatgaacag tttgagagct gaagacactg ccgtttatta ctgtaccagg      300
cgatggaata atttctgggg ccaaggcacc actgtcacag tctcctca      348

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<210> 52
 <211> 321
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J415-5

<400> 52
 aacattgtaa tgaccaat tcccaaatcc atgtccgcct cagcaggaga gaggatgacc 60
 ttgacctgca aggccagtga gaatgtgggt acttatgtgt cctgggtatca acagaaacca 120
 acacagtctc ctaagatgtt gatatacggg gcatccaacc gggtcactgg ggtcccagat 180
 cgcttctccg gcagtggatc tggaacagat ttcattctga ccatcagcag tgtgcaggca 240
 gaagaccttg tagattatta ctgtggacag agttacacct ttccgtacac gttcggaggg 300
 gggaccaagc tggaaatgaa g 321

<210> 53
 <211> 810
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-1

<221> CDS
 <222> (122)...(160)

<221> CDS
 <222> (249)...(608)

<400> 53
 aagcttatga atatgcaaat cctctgaatc tacatggtaa atataggttt gtctatacca 60
 caaacagaaa aacatgagat cacagttctc tctacagtta ctgagcacac aggacctcac 120
 c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gctacaggta 170
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr
 1 5 10

aggggctcac agtagcaggc ttgaggtctg gacatatata tgggtgacaa tgacatccac 230
 ttgtgccttct tctccaca ggt gtc cac tcc gaa gtg aaa ctt gag gag tct 281
 Gly Val His Ser Glu Val Lys Leu Glu Glu Ser
 15 20

gga gga ggc ttg gtg caa cct gga ggg tcc atg aaa atc tcc tgt aaa 329
 Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Met Lys Ile Ser Cys Lys
 25 30 35 40

gcc tct gga ttc act ttc agt aat tac tgg atg aac tgg gtc cgc cag 377
 Ala Ser Gly Phe Thr Phe Ser Asn Tyr Trp Met Asn Trp Val Arg Gln
 45 50 55

act cca gag aag ggg ctt gag tgg gtt gct ctt att aga tgc caa tct 425
 Thr Pro Glu Lys Gly Leu Glu Trp Val Ala Leu Ile Arg Ser Gln Ser
 60 65 70

aat aat ttt gca aca cat tat gcg gag tct gtg aaa ggg agg gtc atc 473
 Asn Asn Phe Ala Thr His Tyr Ala Glu Ser Val Lys Gly Arg Val Ile
 75 80 85

atc tca aga gat gat tcc aag agt agt gtc tac ctg caa atg aac agt 521
 Ile Ser Arg Asp Asp Ser Lys Ser Ser Val Tyr Leu Gln Met Asn Ser

90	95	100	
ttg aga gct gaa gac act gcc gtt tat tac tgt acc agg cga tgg aat			569
Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Arg Trp Asn			
105	110	115	120
aat ttc tgg ggc caa ggc acc act gtc aca gtc tcc tca ggtgagtcct			618
Asn Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser			
	125	130	
tacaacctct ctcttctatt cagcttaaata agattttact gcattttgttg ggggggaaat			678
gtgtgtatct gaatttcagg tcatgaagga ctaggacac cttgggagtc agaaagggtc			738
attgggagcc cgggctgatg cagacagaca tctcagctc ccagacttca tggccagaga			798
tttataggat cc			810

<210> 54
 <211> 133
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-1

<400> 54	
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Gly Val His	
1 5 10 15	
Ser Glu Val Lys Leu Glu Glu Ser Gly Gly Leu Val Gln Pro Gly	
20 25 30	
Gly Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Phe Thr Phe Ser Asn	
35 40 45	
Tyr Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp	
50 55 60	
Val Ala Leu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala	
65 70 75 80	
Glu Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser	
85 90 95	
Ser Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val	
100 105 110	
Tyr Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr	
115 120 125	
Val Thr Val Ser Ser	
130	

<210> 55
 <211> 810
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-1

<400> 55	
ggatcctata aatctctggc catgaagtct gggagctgag gatgtctgtc tgcacagcc	60
cgggctccca atgacccttt ctgactccca aggtgtccct agtccttcac gacctgaaat	120
tcagatacac acatttcccc cccaacaaat gcagtaaaat ctatttaagc tgaatagaag	180
agagaggttg taaggactca cctgaggaga ctgtgacagt ggtgccttgg cccagaaat	240
tattccatcg cctggtacag taataaacgg cagtgtcttc agctctcaaa ctgttcattt	300
gcaggtagac actactcttg gaatcatctc ttgagatgat gacctccct ttcacagact	360
ccgcataatg tggtgcaaaa ttattagatt gcgatctaata aagagcaacc cactcaagcc	420
ccttctctgg agtctggcgg acccagttca tccagtaatt actgaaagtg aatccagagg	480
ctttacagga gattttcatg gacctccag gttgcaccaa gcctcctcca gactcctcaa	540

gtttcacttc	ggagtggaca	cctgtggaga	gaaaggcaaa	gtggatgtca	ttgtcaccca	600
tatatatgtc	cagacctcaa	gcctgctact	gtgagcccct	tacctgtagc	tgttgctacc	660
aagaagagga	tgatacagct	ccatcccatg	gtgaggtcct	gtgtgctcag	taactgtaga	720
gagaactgtg	atctcatgtt	tttctgtttg	tggtatagac	aaacctatat	ttaccatgta	780
gattcagagg	atttgcata	tcataagctt				810

<210> 56
 <211> 620
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J415-1

<221> CDS
 <222> (122) ... (160)

<221> CDS
 <222> (249) ... (581)

<400> 56	
aagcttatga	atatgcaa
aatcctga	atc
tacatggtaa	atatagggtt
gtctatacca	
caaacagaaa	aacatgagat
cacagttctc	tctacagtta
ctgagcacac	aggacctcac
c atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gctacaggta	170
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr	
1 5 10	

aggggctcac	agtagcaggc	ttgaggtctg	gacatatata	tgggtgacaa	tgacatccac	230
tttgcccttc	tctocaca	ggg gtc cac tcc aac att gta atg acc caa tcc				281
	Gly Val His Ser Asn Ile Val Met Thr Gln Ser					
	15 20					

ccc aaa tcc atg tcc gcc tca gca gga gag agg atg acc ttg acc tgc	329
Pro Lys Ser Met Ser Ala Ser Ala Gly Glu Arg Met Thr Leu Thr Cys	
25 30 35 40	

aag gcc agt gag aat tcc ggt act tat gtg tcc tgg tat caa cag aaa	377
Lys Ala Ser Glu Asn Ser Gly Thr Tyr Val Ser Trp Tyr Gln Gln Lys	
45 50 55	

cca aca cag tct cct aag atg ttg ata tac ggg gca tcc aac cgg ttc	425
Pro Thr Gln Ser Pro Lys Met Leu Ile Tyr Gly Ala Ser Asn Arg Phe	
60 65 70	

act ggg gtc cca gat cgc ttc tcc ggc agt gga tct gga aca gat ttc	473
Thr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe	
75 80 85	

att ctg acc gcc agc agt gtg cag gca gaa gac cct gta gat tat tac	521
Ile Leu Thr Ala Ser Ser Val Gln Ala Glu Asp Pro Val Asp Tyr Tyr	
90 95 100	

tgt gga cag agt tac acc ttt ccg tac acg ttc gga ggg ggg acc aag	569
Cys Gly Gln Ser Tyr Thr Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys	
105 110 115 120	

ctg gaa atg aag cgtgagtaga atttaaactt tgcttccctca gttggatcc	620
Leu Glu Met Lys	

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<210> 57
 <211> 124
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J415-1

<400> 57
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Gly Val His
 1 5 10 15
 Ser Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala
 20 25 30
 Gly Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Ser Gly Thr
 35 40 45
 Tyr Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu
 50 55 60
 Ile Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser
 65 70 75 80
 Gly Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln
 85 90 95
 Ala Glu Asp Pro Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro
 100 105 110
 Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 115 120

<210> 58
 <211> 620
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J415-1

<400> 58
 ggatccaact gaggaagcaa agtttaaatt ctactcaagc ttcatttcca gcttggtccc 60
 ccctccgaac gtgtacggaa aggtgtaact ctgtccacag taataatcta caggggtcttc 120
 tgctgcaca ctgctggcgg tcagaatgaa atctgttcca gatccactgc cggagaagcg 180
 atctgggacc ccagtgaacc gggtggatgc cccgtatatc aacatcttag gagactgtgt 240
 tggtttctgt tgataccagg acacataagt accggaattc tcaactggcct tgcaggtcaa 300
 ggtcatcctc tctcctgctg aggcggacat ggatttgggg gattgggtca ttacaatgtt 360
 ggagtggaca cctgtggaga gaaaggcaaa gtggatgtca ttgtcaccca tatatatgtc 420
 cagacctcaa gcctgctact gtgagccct tacctgtagc tgttgctacc aagaagagga 480
 tgatacagct ccatcccatg gtgaggtcct gtgtgctcag taactgtaga gagaactgtg 540
 atctcatgtt tttctgtttg tggatatagac aaacctatat ttaccatgta gattcagagg 600
 atttgcataat tcataagctt 620

<210> 59
 <211> 116
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-2

<400> 59
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val

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      35              40              45
Ala Leu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
      50              55              60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Ser Lys Ser Ser
      65              70              75              80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
      85              90              95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
      100              105              110
Thr Val Ser Ser
      115

```

<210> 60
 <211> 116
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized heavy chain J415-3

```

<400> 60
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1              5              10              15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
      20              25              30
Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val
      35              40              45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
      50              55              60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
      65              70              75              80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
      85              90              95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
      100              105              110
Thr Val Ser Ser
      115

```

<210> 61
 <211> 116
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> majority sequence

```

<400> 61
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1              5              10              15
Ser Met Lys Ile Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
      20              25              30
Trp Met Asn Trp Val Arg Gln Thr Pro Glu Lys Gly Leu Glu Trp Val
      35              40              45
Ala Glu Ile Arg Ser Gln Ser Asn Asn Phe Ala Thr His Tyr Ala Glu
      50              55              60
Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
      65              70              75              80
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
      85              90              95
Tyr Cys Thr Arg Arg Trp Asn Asn Phe Trp Gly Gln Gly Thr Thr Val
      100              105              110

```

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Thr Val Ser Ser
115

<210> 62
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-2

<400> 62
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Pro Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 63
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-3

<400> 63
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly
1 5 10 15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
20 25 30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
35 40 45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ala Ser Ser Val Gln Ala
65 70 75 80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
85 90 95
Thr Phe Gly Gly Thr Lys Leu Glu Met Lys
100 105

<210> 64
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> deimmunized light chain J415-4

<400> 64
Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Ala Ser Ala Gly

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```

      1             5             10             15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
      20             25             30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
      35             40             45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
      50             55             60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
      65             70             75             80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
      85             90             95
Thr Phe Gly Gly Thr Lys Leu Glu Met Lys
      100             105

```

<210> 65

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-6

<400> 65

```

Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
      1             5             10             15
Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
      20             25             30
Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
      35             40             45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
      50             55             60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
      65             70             75             80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
      85             90             95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
      100             105

```

<210> 66

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> deimmunized light chain J415-7

<400> 66

```

Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
      1             5             10             15
Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
      20             25             30
Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
      35             40             45
Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
      50             55             60
Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
      65             70             75             80
Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
      85             90             95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
      100             105

```

<210> 67
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> deimmunized light chain J415-8

<400> 67
 Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1 5 10 15
 Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Ser Gly Thr Tyr
 20 25 30
 Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Met Leu Ile
 35 40 45
 Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
 65 70 75 80
 Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 100 105

<210> 68
 <211> 107
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> majority sequence

<400> 68
 Asn Ile Val Met Thr Gln Phe Pro Lys Ser Met Ser Ala Ser Ala Gly
 1 5 10 15
 Glu Arg Met Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Gly Thr Tyr
 20 25 30
 Val Ser Trp Tyr Gln Gln Lys Pro Thr Gln Ser Pro Lys Met Leu Ile
 35 40 45
 Tyr Gly Ala Ser Asn Arg Phe Thr Gly Val Pro Asp Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr Ile Ser Ser Val Gln Ala
 65 70 75 80
 Glu Asp Leu Val Asp Tyr Tyr Cys Gly Gln Ser Tyr Thr Phe Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Met Lys
 100 105

<210> 69
 <211> 123
 <212> PRT
 <213> Mus musculus

<400> 69
 Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
 35 40 45

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Ala Glu Ile Arg Leu Lys Ser Asp Asn Tyr Ala Thr His Tyr Ala Glu
 50 55 60
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
 65 70 75 80
 Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
 85 90 95
 Tyr Cys Thr Thr Gly Gly Tyr Gly Gly Arg Arg Ser Trp Phe Ala Tyr
 100 105 110
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 70

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> majority sequence

<400> 70

Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Glu Ile Arg Leu Gln Ser Asp Asn Phe Ala Thr His Tyr Ala Glu
 50 55 60
 Ser Val Lys Gly Arg Val Ile Ile Ser Arg Asp Asp Ser Lys Ser Ser
 65 70 75 80
 Val Tyr Leu Gln Met Asn Asn Leu Arg Ala Glu Asp Thr Gly Ile Tyr
 85 90 95
 Tyr Cys Thr Thr Gly Gly Tyr Gly Gly Arg Arg Ser Trp Asn Ala Phe
 100 105 110
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 71

<211> 113

<212> PRT

<213> Mus musculus

<400> 71

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
 1 5 10 15
 Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
 20 25 30
 Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45
 Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60
 Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80
 Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 85 90 95
 Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
 100 105 110
 Lys

<210> 72

<211> 113
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> majority sequence

<400> 72
 Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
 1 5 10 15
 Glu Lys Val Thr Leu Ser Cys Lys Ala Ser Glu Ser Leu Leu Asn Val
 20 25 30
 Gly Asn Gln Lys Thr Tyr Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45
 Ser Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60
 Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Ile Leu Thr
 65 70 75 80
 Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gly Asn
 85 90 95
 Ser Tyr Ser Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu
 100 105 110
 Lys

<210> 73
 <211> 354
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(354)

<400> 73
 gag gtc cag ctg cag cag tct gga cct gag ctg gtt aag cct ggg gct 48
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 tca gtg aag atg tcc tgc aag gct tct gga tac aca ttc act ggc tat 96
 Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30
 gtt atg cac tgg gtg aag cag aag cct gga cag gtc ctt gag tgg att 144
 Val Met His Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile
 35 40 45
 gga tat att aat cct tac aat gat gtt act agg tat aat ggg aag ttc 192
 Gly Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe
 50 55 60
 aaa ggc aag gcc aca ctg acc tca gac aaa tat tcc agc aca gcc tac 240
 Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr
 65 70 75 80
 atg gag ctc agc ggc ctg acc tct gag gac tct gcg gtc tat tac tgt 288
 Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 gca aga ggg gag aac tgg tac tac ttt gac tcc tgg ggc cga ggc gcc 336
 Ala Arg Gly Glu Asn Trp Tyr Tyr Phe Asp Ser Trp Gly Arg Gly Ala

100 105 110 354

act ctc aca gtc tcc tca
Thr Leu Thr Val Ser Ser
115

<210> 74
<211> 118
<212> PRT
<213> Mus musculus

<400> 74
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20 25 30
Val Met His Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile
35 40 45
Gly Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe
50 55 60
Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Arg Gly Glu Asn Trp Tyr Tyr Phe Asp Ser Trp Gly Arg Gly Ala
100 105 110
Thr Leu Thr Val Ser Ser
115

<210> 75
<211> 354
<212> DNA
<213> Mus musculus

<400> 75
tgaggagact gtgagagtgg cgctcggccc ccaggagtca aagtagtacc agttctcccc 60
tcttgacacag taatagaccg cagagtcctc agaggtcagg ccgctgagct ccatgtaggc 120
tgtgctggaa tatttgctcg aggtcagtggt ggccttgccct ttgaacttcc cattatacct 180
agtaacatca ttgtaaggat taatataatcc aatccactca aggacctgtc caggcttctg 240
cttcacccag tgcataacat agccagtga tgtgtatcca gaagccttgc aggacatctt 300
cactgaagcc ccaggcttaa ccagctcagg tccagactgc tgcagctgga cctc 354

<210> 76
<211> 333
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(333)

<400> 76
gac att gtg ctg acc caa tct cca gct tct ttg gct gtg tct cta gga 48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15
cag agg gcc acc ata tcc tgc aga gcc agt gaa agt att gat agt tat 96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Ile Asp Ser Tyr
20 25 30

gac aat act ttt atg cac tgg tac cag cag aaa cca gga cag cca ccc 144
 Asp Asn Thr Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45

aac ctc ctc atc ttt cgt gca tcc atc cta gaa tct ggg atc cct gcc 192
 Asn Leu Leu Ile Phe Arg Ala Ser Ile Leu Glu Ser Gly Ile Pro Ala
 50 55 60

agg ttc agt ggc agt ggg tct ggg aca gac ttc acc ctc acc att tat 240
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr
 65 70 75 80

cct gtg gag gct gat gat gtt gca acc tat tac tgt cac caa agt att 288
 Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys His Gln Ser Ile
 85 90 95

gag gat ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa 333
 Glu Asp Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 77
 <211> 111
 <212> PRT
 <213> Mus musculus

<400> 77
 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Ile Asp Ser Tyr
 20 25 30
 Asp Asn Thr Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Asn Leu Leu Ile Phe Arg Ala Ser Ile Leu Glu Ser Gly Ile Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr
 65 70 75 80
 Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys His Gln Ser Ile
 85 90 95
 Glu Asp Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 78
 <211> 333
 <212> DNA
 <213> Mus musculus

<400> 78
 ttttatttcc agcttggtcc cccctccgaa cgtgtacgga tcctcaatac tttggtgaca 60
 gtaatagggtt gcaacatcat cagcctccac aggataaatg gtgagggtga agtctgtccc 120
 agaccactg ccaactgaacc tggcagggat cccagattct aggatggatg cacgaaagat 180
 gaggaggttg ggtggctgtc ctggtttctg ctggtaccag tgcataaaag tattgtcata 240
 actatcaata ctttactggt ctctgcagga tatggtggcc ctctgtccta gagacacagc 300
 caaagaagct ggagattggg tcagacaaat gtc 333

<210> 79
 <211> 125
 <212> PRT
 <213> Mus musculus

<400> 79

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30
 Tyr Met Asn Asn Trp Val Lys Gln Ser Pro Gly Lys Ser Leu Glu Trp
 35 40 45
 Ile Gly Asp Ile Asn Pro Gly Asn Gly Gly Thr Ser Tyr Asn Gln Lys
 50 55 60
 Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
 65 70 75 80
 Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Gly Tyr Tyr Ser Ser Ser Tyr Met Ala Tyr Tyr Ala Phe
 100 105 110
 Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120 125

<210> 80
 <211> 125
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> majority sequence

<400> 80
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30
 Val Met Asn Asn Trp Val Lys Gln Ser Pro Gly Gln Val Leu Glu Trp
 35 40 45
 Ile Gly Asp Ile Asn Pro Gly Asn Gly Gly Thr Ser Tyr Asn Gly Lys
 50 55 60
 Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala
 65 70 75 80
 Tyr Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Gly Glu Asn Ser Ser Ser Tyr Met Ala Tyr Tyr Ala Phe
 100 105 110
 Asp Ser Trp Gly Gln Gly Ala Thr Val Thr Val Ser Ser
 115 120 125

<210> 81
 <211> 112
 <212> PRT
 <213> Mus musculus

<400> 81
 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr
 20 25 30
 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80
 Pro Val Glu Glu Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn
 85 90 95

Glu Asp Pro Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 82
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> majority sequence

<400> 82
 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr
 20 25 30
 Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45
 Asn Leu Leu Ile Phe Ala Ala Ser Ile Leu Glu Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His
 65 70 75 80
 Pro Val Glu Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Ile
 85 90 95
 Glu Asp Pro Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 83
 <211> 363
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(363)

<400> 83
 cag gtg cag cta aag gag tca gga cct ggc ctg gtg gcg tcc tca cag 48
 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1 5 10 15
 agc ctg tcc atc aca tgc acc gtc tca gga ttc tca tta acc gcc tat 96
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
 20 25 30
 ggt att aac tgg gtt cgc cag cct cca gga aag ggt ctg gag tgg ctg 144
 Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 gga gtg ata tgg cct gat gga aac aca gac tat aat tca act ctc aaa 192
 Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
 50 55 60
 tcc aga ctg aac atc ttc aag gac aac tcc aag aac caa gtt ttc tta 240
 Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
 65 70 75 80
 aaa atg agc agt ttc caa act gat gac aca gcc aga tac ttc tgt gcc 288
 Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
 85 90 95

30/39

aga gat tgc tat ggt aac ttc aag agg ggt tgg ttt gac ttc tgg ggc 336
 Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
 100 105 110

cag ggc acc act ctc aca gtc tcc tca 363
 Gln Gly Thr Thr Leu Thr Val Ser Ser
 115 120

<210> 84
 <211> 121
 <212> PRT
 <213> Mus musculus

<400> 84
 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
 20 25 30
 Gly Ile Asn Trp Val Arg Gln Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45
 Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
 50 55 60
 Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
 65 70 75 80
 Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
 85 90 95
 Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
 100 105 110
 Gln Gly Thr Thr Leu Thr Val Ser Ser
 115 120

<210> 85
 <211> 363
 <212> DNA
 <213> Mus musculus

<400> 85
 tgaggagact gtgagagtgg tgccttggcc ccagaagtca aaccaacccc tcttgaagtt 60
 accatacgaa tctctggcac agaagtatct ggctgtgtca tcagtttggg aactgctcat 120
 ttttaagaaa acttggttct tggagttgtc cttgaagatg ttcagtctgg atttgagagt 180
 tgaattatag tctgtgtttc catcaggcca tatcactccc agccactcca gaccctttcc 240
 tggaggctgg cgaacccagt taataccata ggcggttaat gagaatcctg agacggtgca 300
 tgtgatggac aggtctctgtg aggacgccac caggccaggt cctgactcct ttagctgcac 360
 ctg 363

<210> 86
 <211> 321
 <212> DNA
 <213> Mus musculus

<220>
 <221> CDS
 <222> (1)...(321)

<400> 86
 aac att gtg atg acc cag tct caa aaa ttc atg tcc aca tca cca gga 48
 Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly
 1 5 10 15
 gac agg gtc agg gtc acc tgc aag gcc agt cag aat gtg ggt tct gat 96

Asp	Arg	Val	Arg	Val	Thr	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Ser	Asp	
		20						25					30			
gta	gcc	tgg	tat	caa	gcg	aaa	cca	gga	caa	tct	cct	aga	ata	ctg	att	144
Val	Ala	Trp	Tyr	Gln	Ala	Lys	Pro	Gly	Gln	Ser	Pro	Arg	Ile	Leu	Ile	
		35					40				45					
tac	tcg	aca	tcc	tac	cgt	tac	agt	ggg	gtc	cct	gat	cgc	ttc	aca	gcc	192
Tyr	Ser	Thr	Ser	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Ala	
		50				55				60						
tat	gga	tct	ggg	aca	gat	ttc	act	ctc	acc	att	acc	aat	gtg	cag	tct	240
Tyr	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Thr	Asn	Val	Gln	Ser	
		65			70				75					80		
gaa	gac	ttg	aca	gag	tat	ttc	tgt	cag	caa	tat	aat	agc	tat	cct	ctc	288
Glu	Asp	Leu	Thr	Glu	Tyr	Phe	Cys	Gln	Gln	Tyr	Asn	Ser	Tyr	Pro	Leu	
			85					90					95			
acg	ttc	ggt	gct	ggg	acc	aag	ctg	gag	ctg	aaa						321
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys						
		100						105								

<210> 87
 <211> 107
 <212> PRT
 <213> Mus musculus

Asn	Ile	Val	Met	Thr	Gln	Ser	Gln	Lys	Phe	Met	Ser	Thr	Ser	Pro	Gly	
1			5						10					15		
Asp	Arg	Val	Arg	Val	Thr	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Ser	Asp	
		20						25					30			
Val	Ala	Trp	Tyr	Gln	Ala	Lys	Pro	Gly	Gln	Ser	Pro	Arg	Ile	Leu	Ile	
		35					40					45				
Tyr	Ser	Thr	Ser	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Ala	
		50				55				60						
Tyr	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Thr	Asn	Val	Gln	Ser	
		65			70				75					80		
Glu	Asp	Leu	Thr	Glu	Tyr	Phe	Cys	Gln	Gln	Tyr	Asn	Ser	Tyr	Pro	Leu	
			85					90					95			
Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys						
		100						105								

<210> 88
 <211> 321
 <212> DNA
 <213> Mus musculus

tttcagctcc	agcttgggtcc	cagcaccgaa	cgtgagagga	tagctattat	attgctgaca	60
gaaatactct	gtcaagtctt	cagactgcac	attggtaatg	gtgagagtga	aatctgtccc	120
agatccatag	gctgtgaagc	gatcagggac	cccactgtaa	cggtaggatg	tcgagtaaat	180
cagtattcta	ggagattgtc	ctggtttcgc	ttgataccag	gctacatcag	aaccacatt	240
ctgactggcc	ttgcaggtga	ccctgaccct	gtctctgggt	gatgtggaca	tgaatttttg	300
agactgggtc	atcacaatgt	t				321

<210> 89
 <211> 121
 <212> PRT

<213> Mus musculus

<400> 89

```

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1           5           10           15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
          20           25           30
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
          35           40           45
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
          50           55           60
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
          65           70           75           80
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
          85           90           95
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
          100          105          110
Gln Gly Thr Thr Leu Thr Val Ser Ser
          115          120

```

<210> 90

<211> 121

<212> PRT

<213> Artificial Sequence

<220>

<223> majority sequence

<400> 90

```

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1           5           10           15
Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ala Tyr
          20           25           30
Gly Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu
          35           40           45
Gly Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys
          50           55           60
Ser Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu
          65           70           75           80
Lys Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala
          85           90           95
Arg Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe Trp Gly
          100          105          110
Gln Gly Thr Thr Leu Thr Val Ser Ser
          115          120

```

<210> 91

<211> 113

<212> PRT

<213> Mus musculus

<400> 91

```

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
 1           5           10           15
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
          20           25           30
Gly Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
          35           40           45
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
          50           55           60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

```

```
<210> 92
<211> 115
<212> PRT
<213> Artificial Sequence
```

<220>
<223> majority sequence

```

<400> 92
Asp Ile Val Met Thr Gln Ser Gln Ser Ser Leu Ala Val Ser Ala Gly
 1              5              10              15
Asp Lys Val Thr Val Ser Cys Lys Ala Ser Gln Ser Leu Leu Asn Val
      20              25              30
Gly Ser Asp Lys Asn Tyr Val Ala Trp Tyr Gln Ala Lys Pro Gly Gln
      35              40              45
Ser Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Arg Glu Ser Gly Val
      50              55              60
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65              70              75              80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Asn
      85              90              95
Asp Asn Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
      100              105              110
Lys Arg Ala
      115

```

```
<210> 93
<211> 10
<212> PRT
<213> Mus musculus
```

```
<400> 93
Gly Tyr Thr Phe Thr Gly Tyr Val Met His
 1             5             10
```

```
<210> 94
<211> 17
<212> PRT
<213> Mus musculus
```

```

<400> 94
Tyr Ile Asn Pro Tyr Asn Asp Val Thr Arg Tyr Asn Gly Lys Phe Lys
 1             5             10             15
Gly

```

```
<210> 95
<211> 9
<212> PRT
<213> Mus musculus
```

<400> 95
Gly Glu Asn Trp Tyr Tyr Phe Asp Ser

1

5

<210> 96
<211> 15
<212> PRT
<213> Mus musculus

<400> 96
Arg Ala Ser Glu Ser Ile Asp Ser Tyr Asp Asn Thr Phe Met His
1 5 10 15

<210> 97
<211> 7
<212> PRT
<213> Mus Musculus

<400> 97
Arg Ala Ser Ile Leu Glu Ser
1 5

<210> 98
<211> 9
<212> PRT
<213> Mus musculus

<400> 98
His Gln Ser Ile Glu Asp Pro Tyr Thr
1 5

<210> 99
<211> 10
<212> PRT
<213> Mus musculus

<400> 99
Gly Phe Ser Leu Thr Ala Tyr Gly Ile Asn
1 5 10

<210> 100
<211> 16
<212> PRT
<213> Mus musculus

<400> 100
Val Ile Trp Pro Asp Gly Asn Thr Asp Tyr Asn Ser Thr Leu Lys Ser
1 5 10 15

<210> 101
<211> 13
<212> PRT
<213> Mus musculus

<400> 101
Asp Ser Tyr Gly Asn Phe Lys Arg Gly Trp Phe Asp Phe
1 5 10

<210> 102
<211> 11
<212> PRT
<213> Mus musculus

<400> 102

Lys Ala Ser Gln Asn Val Gly Ser Asp Val Ala
1 5 10

<210> 103

<211> 7

<212> PRT

<213> Mus musculus

<400> 103

Ser Thr Ser Tyr Arg Tyr Ser
1 5

<210> 104

<211> 9

<212> PRT

<213> Mus musculus

<400> 104

Gln Gln Tyr Asn Ser Tyr Pro Leu Thr
1 5

<210> 105

<211> 25

<212> PRT

<213> Mus musculus

<400> 105

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Met Ser Cys Lys Ala Ser
20 25

<210> 106

<211> 14

<212> PRT

<213> Mus musculus

<400> 106

Trp Val Lys Gln Lys Pro Gly Gln Val Leu Glu Trp Ile Gly
1 5 10

<210> 107

<211> 32

<212> PRT

<213> Mus musculus

<400> 107

Lys Ala Thr Leu Thr Ser Asp Lys Tyr Ser Ser Thr Ala Tyr Met Glu
1 5 10 15
Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 108

<211> 11

<212> PRT

<213> Mus musculus

<400> 108

Trp Gly Arg Gly Ala Thr Leu Thr Val Ser Ser
1 5 10

```

<400> 109
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1          5          10          15
Ser Val Lys Met Ser Cys Lys Ala Ser Trp Val Lys Gln Lys Pro Gly
 20          25          30
Gln Val Leu Glu Trp Ile Gly Lys Ala Thr Leu Thr Ser Asp Lys Tyr
 35          40          45
Ser Ser Thr Ala Tyr Met Glu Leu Ser Gly Leu Thr Ser Glu Asp Ser
 50          55          60
Ala Val Tyr Tyr Cys Ala Arg Trp Gly Arg Gly Ala Thr Leu Thr Val
 65          70          75          80
Ser Ser

```

```
<210> 110
<211> 23
<212> PRT
<213> Mus musculus
```

```
<400> 110
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1             5             10             15
Gln Arg Ala Thr Ile Ser Cys
      20
```

```
<210> 111
<211> 15
<212> PRT
<213> Mus musculus
```

<400> 111
Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Asn Leu Leu Ile Phe
1 5 10 15

```
<210> 112
<211> 32
<212> PRT
<213> Mus musculus
```

```

<400> 112
Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 1             5             10             15
Leu Thr Ile Tyr Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys
      20             25             30

```

```
<210> 113
<211> 10
<212> PRT
<213> Mus musculus
```

<400> 113
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
1 5 10

<210> 114

<211> 80
 <212> PRT
 <213> Mus musculus

<400> 114
 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Trp Tyr Gln Gln Lys Pro Gly Gln Pro
 20 25 30
 Pro Asn Leu Leu Ile Phe Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
 35 40 45
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Tyr Pro Val Glu Ala Asp Asp
 50 55 60
 Val Ala Thr Tyr Tyr Cys Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 65 70 75 80

<210> 115
 <211> 25
 <212> PRT
 <213> Mus musculus

<400> 115
 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser
 20 25

<210> 116
 <211> 14
 <212> PRT
 <213> Mus musculus

<400> 116
 Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly
 1 5 10

<210> 117
 <211> 32
 <212> PRT
 <213> Mus musculus

<400> 117
 Arg Leu Asn Ile Phe Lys Asp Asn Ser Lys Asn Gln Val Phe Leu Lys
 1 5 10 15
 Met Ser Ser Phe Gln Thr Asp Asp Thr Ala Arg Tyr Phe Cys Ala Arg
 20 25 30

<210> 118
 <211> 11
 <212> PRT
 <213> Mus musculus

<400> 118
 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
 1 5 10

<210> 119
 <211> 82
 <212> PRT
 <213> Mus musculus

<400> 119

Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Ser Ser Gln
 1 5 10 15
 Ser Leu Ser Ile Thr Cys Thr Val Ser Trp Val Arg Gln Pro Pro Gly
 20 25 30
 Lys Gly Leu Glu Trp Leu Gly Arg Leu Asn Ile Phe Lys Asp Asn Ser
 35 40 45
 Lys Asn Gln Val Phe Leu Lys Met Ser Ser Phe Gln Thr Asp Asp Thr
 50 55 60
 Ala Arg Tyr Phe Cys Ala Arg Trp Gly Gln Gly Thr Thr Leu Thr Val
 65 70 75 80
 Ser Ser

<210> 120

<211> 23

<212> PRT

<213> Mus musculus

<400> 120

Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly
 1 5 10 15
 Asp Arg Val Arg Val Thr Cys
 20

<210> 121

<211> 15

<212> PRT

<213> Mus musculus

<400> 121

Trp Tyr Gln Ala Lys Pro Gly Gln Ser Pro Arg Ile Leu Ile Tyr
 1 5 10 15

<210> 122

<211> 32

<212> PRT

<213> Mus musculus

<400> 122

Gly Val Pro Asp Arg Phe Thr Ala Tyr Gly Ser Gly Thr Asp Phe Thr
 1 5 10 15
 Leu Thr Ile Thr Asn Val Gln Ser Glu Asp Leu Thr Glu Tyr Phe Cys
 20 25 30

<210> 123

<211> 10

<212> PRT

<213> Mus musculus

<400> 123

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 1 5 10

<210> 124

<211> 80

<212> PRT

<213> Mus musculus

<400> 124

Asn Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Pro Gly

1	5	10	15
Asp Arg Val	Arg Val Thr Cys Trp	Tyr Gln Ala Lys Pro	Gly Gln Ser
	20	25	30
Pro Arg Ile	Leu Ile Tyr Gly Val	Pro Asp Arg Phe Thr	Ala Tyr Gly
	35	40	45
Ser Gly Thr	Asp Phe Thr Leu Thr	Ile Thr Asn Val	Gln Ser Glu Asp
	50	55	60
Leu Thr Glu	Tyr Phe Cys Phe Gly	Ala Gly Thr Lys	Leu Glu Leu Lys
65	70	75	80

<210> 125

<211> 348

<212> DNA

<213> Mus musculus

<400> 125

gaagtgaagc	ttgaggagtc	tggaggaggc	ttggtgcaac	ctggaggatc	catgaaactc	60
tctctgtgtg	cctctggatt	cactttcagt	aattactgga	tgaactgggt	ccgccagtct	120
ccagagaagc	ggcttgagtg	ggttgctgaa	attagatcgc	aatctaataa	ttttgcaaca	180
cattatgcgc	agtctgtgaa	agggagggtc	atcatctcaa	gagatgattc	caagagtagt	240
gtctacctgc	aaatgaacaa	cttgagagct	gaagacactg	gcatttatta	ctgtaccagg	300
cgatggaata	atttctgggg	ccaaggcacc	actctcacag	tctctctca		348

<210> 126

<211> 348

<212> DNA

<213> Mus musculus

<400> 126

tgaggagact	gtgagagtgg	tgccttggcc	ccagaaatta	ttccatcgcc	tggtagacga	60
ataaatgcca	gtgtcttcag	ctctcaagtt	gttcatttgc	aggtagacac	tactcttgga	120
atcatctctt	gagatgatga	ccctcccttt	cacagactcc	gcataatgtg	ttgcaaaaatt	180
attagattgc	gatctaattt	cagcaaccca	ctcaagcccc	ttctctggag	actggcggac	240
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<213> Mus musculus

<400> 128

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aaattgggtc	attacaatgt	t				321



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5510 Telex: 079-065 ATCCNORTH • FAX: 301-770-2517

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Laboratory of Urological Oncology
Attn: Neil H. Bander, M.D.
Cornell Medical Center - Box 23
1300 York Avenue
New York, NY 10021

Deposited on Behalf of: Cornell University (c/o Dr. Neil H. Bander)

Identification Reference by Depositor:

ATCC Designation

Mouse hybridoma Prost E89

HB-12101

The deposits were accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposits were received ☒ by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

☒ We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested May 7, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey
Barbara M. Hailey, Administrator, Patent Depository

Date: May 8, 1996

cc: Michael L. Goldman
Lauren S. Stich



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Cornell Medical Center
Attn: Dr. Neil H. Bander or Ms. Lauren Stich
Department of Urology
525 East 68 Street (Box 23, Rm. E-300)
New York, NY 10021

Deposited on Behalf of: Cornell Medical Center

Identification Reference by Depositor:

ATCC Designation

Mouse hybridoma prost J415

HB-12109

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposit was received May 30, 1996 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: ☒ We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 5, 1996. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey
Barbara M. Hailey, Administrator, Patent Depository

Date: June 5, 1996

cc: ☒ Michael L. Goldman
H. Walter Haeussler

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

**BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE**

INTERNATIONAL FORM

**RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2**

To: (Name and Address of Depositor or Attorney)

Weill Medical College of Cornell University
Laboratory of Urological Oncology
Attn: Neil H. Bander
E-300
1300 York Avenue
New York, NY 10021

Deposited on Behalf of: Weill Medical College of Cornell University (Cornell Research Foundation)

Identification Reference by Depositor:

Patent Deposit Designation

Murine NSO myeloma cell line: 109BS J415 DIVH4 DIVK5

PTA-4174

The deposit was accompanied by: a scientific description a proposed taxonomic description indicated above.

The deposit was received March 21, 2002 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested April 3, 2002. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Marie Harris
Marie Harris, Patent Specialist, ATCC Patent Depository

Date: April 16, 2002

cc: Louis Myers
(Ref: Docket or Case No.: 10448/163001 MPI01-140P3RCP1)



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301)231-5520 Telex: 908-768 ATCCROVE • FAX: (301)816-4366

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

New York Hospital-Cornell Medical Center
Attn: Neil H. Bander, M.D.
Laboratory of Urological Oncology, Rm. E-300 (Box 23)
525 E. 68 Street
New York, NY 10021

Deposited on Behalf of: Dr. Neil H. Bander, Department of Urology, Cornell Medical Center

Identification Reference by Depositor:

ATCC Designation

Mouse hybridoma Prost J591
Mouse hybridoma Prost J533

HB-12126
HB-12127

The deposits were accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposits were received June 6, 1996 by this International Depository Authority and have been accepted.

AT YOUR REQUEST:

☒ We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested June 12, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey
Barbara M. Hailey, Administrator, Patent Depository

Date: June 13, 1996

cc: ☒ Michael L. Goldman
H. Walter Haeussler

Applicants: Ron S. Israeli et al.
Serial No.: 08/466,381
Filed: June 6, 1995

Pending Claims 97-103

97. A method of detecting micrometastatic prostate tumor cells in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) contacting the mRNA sample under hybridizing conditions with a labeled nucleic acid probe which:
(1) is at least 15 nucleotides in length and (2) hybridizes specifically to a nucleic acid having a sequence which is complementary to a sequence present in the sequence set forth in SEQ ID NO. 1.
- c) removing any unbound labeled nucleic acid probe; and
- d) detecting the presence of labeled nucleic acid probe hybridized to the mRNA;
- e) comparing the amount of labeled nucleic acid probe measured in step d) with an amount measured in a negative control sample which does not have micrometastatic prostate tumor cells, wherein a higher amount measured in step d) compared to the amount measured in the control sample indicates the detection of micrometastatic prostate tumor cells in the subject.

98. A method of detecting micrometastatic prostate tumor cells in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) reverse transcribing the mRNA to generate a single-stranded cDNA;

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Filed: June 6, 1995

- c) contacting the single-stranded cDNA under hybridizing conditions with a labeled nucleic acid probe which: 1) is at least 15 nucleotides in length; and 2) hybridizes specifically to a nucleic acid having a sequence set forth in SEQ ID NO:1;
- d) removing any unbound labeled nucleic acid probe; and
- e) detecting the presence of labeled nucleic acid probe hybridized to the cDNA;
- f) comparing the amount of labeled nucleic acid probe measured in step e) with an amount measured in a negative control sample which does not have micrometastatic prostate tumor cells, wherein a higher amount measured in step e) compared to the amount measured in the control sample indicates the detection of micrometastatic prostate tumor cells in the subject.

99. A method of detecting micrometastatic prostate tumor cells in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) generating a double-stranded mRNA-cDNA duplex from the mRNA;
- c) contacting the duplex from (b) with one primer having a sequence which is complementary to a portion of the sequence set forth in SEQ ID NO:1 and a second primer having a sequence which comprises a different portion of the sequence set forth in SEQ ID NO:1;

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- d) amplifying the nucleic acid from (c) using a polymerase chain reaction to obtain an amplification product;
- e) contacting the amplification product of (d) under hybridizing conditions with a labeled nucleic acid probe which: 1) is at least 15 nucleotides in length; 2) hybridizes specifically to a nucleic acid having a sequence set forth in SEQ ID NO. 1.;
- f) removing any unbound labeled nucleic acid probe; and
- g) detecting the presence of labeled nucleic acid probe hybridized to the amplification product;
- h) comparing the amount of labeled nucleic acid probe measured in step g) with an amount measured in a negative control sample which does not have micrometastatic prostate tumor cells, wherein a higher amount measured in step g) compared to the amount measured in the control sample indicates the detection of micrometastatic prostate tumor cells in the subject.

100. A method of detecting micrometastatic prostate tumor cells in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) generating a double-stranded mRNA-cDNA duplex from the mRNA;
- c) contacting the duplex from (b) with one primer having a sequence which is complementary to a portion of the sequence set forth in SEQ ID NO:1 and a second primer

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Filed: June 6, 1995

having a sequence which comprises a different portion of the sequence set forth in SEQ ID NO:1;

- d) amplifying the nucleic acid from (c) using a polymerase chain reaction to obtain an amplification product;
- e) contacting the amplification product of (d) under hybridizing conditions with a labeled nucleic acid probe which: 1) is at least 15 nucleotides in length; and 2) hybridizes specifically to a nucleic acid having a sequence complementary to the DNA sequence set forth in SEQ ID NO:1.;
- f) removing any unbound labeled nucleic acid probe; and
- g) detecting the presence of labeled nucleic acid probe hybridized to the amplification product;
- h) comparing the amount of labeled nucleic acid probe measured in step g) with an amount measured in a negative a control sample which does not have micrometastatic prostate tumor cells, wherein a higher amount measured in step g) compared to the amount measured in the control sample indicates the detection of micrometastatic prostate tumor cells in the subject.

101. A method of detecting the presence of a nucleic acid encoding a prostate specific membrane antigen in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) generating a double-stranded cDNA from the mRNA;

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Filed: June 6, 1995

- c) contacting the double-stranded cDNA from (b) with one primer having a sequence which is complementary to a portion of the sequence set forth in SEQ ID NO:1 and a second primer having a sequence which comprises a different portion of the sequence set forth in SEQ ID NO:1;
- d) amplifying the double stranded cDNA using a polymerase chain reaction to obtain an amplification product;
- e) contacting the amplification product of (d) under hybridizing conditions with a labeled nucleic acid probe which 1) is at least 15 nucleotides in length; 2) hybridizes specifically to a nucleic acid having a sequence complementary to the DNA sequence set forth in SEQ ID NO:1.;
- f) removing any unbound labeled nucleic acid probe; and
- g) detecting the presence of labeled nucleic acid probe hybridized to the amplification product so as to thereby detect the presence of a nucleic acid encoding a prostate specific membrane antigen in a subject.

102. A method of detecting the presence of a nucleic acid encoding a prostate specific membrane antigen in a subject which comprises:

- a) obtaining a suitable sample of mRNA from the subject;
- b) generating a double-stranded cDNA from the mRNA;
- c) contacting the double-stranded cDNA from (b) with one primer having a sequence which is complementary to a portion of the sequence set forth in SEQ ID NO:1 and a

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Serial No.: 08/466,381
Filed: June 6, 1995

second primer having a sequence which comprises a different portion of the sequence set forth in SEQ ID NO:1;

- d) amplifying the double stranded cDNA using a polymerase chain reaction to obtain an amplification product;
- e) contacting the amplification product of (d) under hybridizing conditions with a labeled nucleic acid probe which 1) is at least 15 nucleotides in length; 2) hybridizes specifically to a nucleic acid having a sequence set forth in SEQ ID NO:1.;
- f) removing any unbound labeled nucleic acid probe; and
- g) detecting the presence of labeled nucleic acid probe hybridized to the amplification product so as to thereby detect the presence of a nucleic acid encoding a prostate specific membrane antigen in a subject.

103. The method of any one of claims 97-102, wherein the sample is blood, lymph nodes, or bone marrow.

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 08/470,735
Filed: June 6, 1995

Pending Claims 128-144 and 161

128. A purified antibody which binds to a region of an outer membrane domain of prostate specific membrane antigen, which region has within its structure the consecutive amino acid sequence Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35).
129. A purified antibody which binds to a region of an outer membrane domain of prostate specific membrane antigen, which region has within its structure the consecutive amino acid sequence Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36).
130. A purified antibody which binds to a region of an outer membrane domain of prostate specific membrane antigen, which region has within its structure the consecutive amino acid sequence Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37).
131. A purified antibody which binds to a fragment of an outer membrane domain of prostate specific membrane antigen, which fragment has within its structure each of the following amino acid sequences:
- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35);
 - (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36);
 - (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37); and
 - (d) Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO: 38).

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 08/470,735
Filed: June 6, 1995

132. A purified antibody which binds to a fragment of an outer membrane domain of prostate specific membrane antigen, which fragment has within its structure each of the following amino acid sequences:

- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35);
- (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36); and
- (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37).

133. A purified antibody which binds to a fragment of prostate specific membrane antigen, which fragment corresponds to a hydrophilic region of an outer membrane domain of prostate specific membrane antigen, the amino acid sequence of which antigen is set forth in SEQ ID NO:2.

134. The purified antibody of claim 133, wherein the fragment comprises within its structure each of the following consecutive amino acid sequences:

- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35);
- (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36); and
- (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37).

135. A purified antibody which binds to an outer membrane domain of prostate specific membrane antigen, the amino acid sequence of which antigen is set forth in SEQ ID NO:2.

136. A purified antibody which binds to a hydrophilic region of an outer membrane domain of prostate specific membrane antigen, the amino acid sequence of which antigen is set

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U.S. Serial No.: 08/470,735
Filed: June 6, 1995

forth in SEQ ID NO:2.

137. A purified antibody which binds to an outer membrane domain of prostate specific membrane antigen, the amino acid sequence of which antigen is set forth in SEQ ID NO:2, wherein the antibody is capable of binding to the surface of a cell.

138. The purified antibody of any one of claims 128-137, wherein the antibody is a monoclonal antibody.

139. A composition of matter comprising the antibody of any one of claims 128-137 and an agent conjugated to the antibody.

140. The composition of matter of claim 139, wherein the agent is a radioisotope or toxin.

141. A composition comprising a carrier and the composition of matter of claim 139.

142. The composition of matter of claim 141, wherein the agent is a radioisotope or toxin.

143. A composition comprising a carrier and the composition of matter of claim 141.

144. A method of imaging prostate cancer in a subject which comprises administering to the subject the composition of

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U.S. Serial No.: 08/470,735
Filed: June 6, 1995

matter of claim 141, wherein the agent is an imaging agent under conditions permitting formation of a complex between the composition of matter and prostate specific membrane antigen, and obtaining an image of any complex so formed.

161.. A monoclonal antibody having an antigen-binding region specific for the extracellular domain of prostate specific membrane antigen, the amino acid sequence of which antigen is set forth in SEQ ID NO:2.

Applicants: Ron. S. Israeli et al.
U.S. Serial No.: 09/724,026
Filed: November 28, 2000

Pending Claims 90-107

90. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and comprising within its structure consecutive amino acids having the sequence Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO:35), provided that the polypeptide comprises more amino acids than those present in SEQ ID NO:35 and the sequence of the polypeptide is included within SEQ ID NO:2 .
91. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and comprising within its structure consecutive amino acids having the sequence Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO:36).
92. The isolated nucleic acid of claim 91 wherein the polypeptide comprises more amino acids than those present in SEQ ID NO:36 and the sequence of the polypeptide is included within SEQ ID NO:2.
93. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and comprising within its structure consecutive amino acids having the sequence Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO:37).

Applicants: Ron. S. Israeli et al.
U.S. Serial No.: 09/724,026
Filed: November 28, 2000

94. The isolated nucleic acid of claim 93 wherein the polypeptide comprises more amino acids than those present in SEQ ID NO:37 and the sequence of the polypeptide is included within SEQ ID NO:2.
95. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and comprising within its structure consecutive amino acids having the sequence Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO:38).
96. The isolated nucleic acid of claim 95 wherein the polypeptide comprises more amino acids than those present in SEQ ID NO:38 and the sequence of the polypeptide is included within SEQ ID NO:2.
97. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and having within its structure each of the following consecutive amino acid sequences:
- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO:35);
 - (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO:36); and
 - (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO:37).
98. The isolated nucleic acid of claim 97 wherein the polypeptide comprises more amino acids than those present

Applicants: Ron. S. Israeli et al.
U.S. Serial No.: 09/724,026
Filed: November 28, 2000

in SEQ ID NO:35, SEQ ID NO:36 and SEQ ID NO:37 and the sequence of the polypeptide is included within SEQ ID NO:2.

99. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, and having within its structure each of the following consecutive amino acid sequences:

- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO:35);
- (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO:36);
- (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO:37); and
- (d) Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO:38).

100. The isolated nucleic acid of claim 99 wherein the polypeptide comprises more amino acids than those present in SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37 and SEQ ID NO:38 and the sequence of the polypeptide is included within SEQ ID NO:2.

101. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide, wherein the polypeptide is capable of generating antibodies specific to prostate specific membrane antigen, and having within its structure consecutive amino acids having a sequence which is present in SEQ ID NO:2.

102. An isolated nucleic acid which encodes an antigenic

Applicants: Ron. S. Israeli et al.
U.S. Serial No.: 09/724,026
Filed: November 28, 2000

prostate specific membrane antigen polypeptide characterized by antigenicity, which nucleic acid has within its structure at least 15 consecutive nucleotides, the sequence of which is present in SEQ ID NO:1.

103. An isolated nucleic acid which encodes an antigenic prostate specific membrane antigen polypeptide characterized by antigenicity, which nucleic acid has within its structure consecutive nucleotides, the sequence of which is in SEQ ID NO:1 and encodes at least six consecutive amino acids shown in SEQ ID NO:2.

104. The isolated nucleic acid of claim 103 wherein the consecutive nucleotides are in SEQ ID NO:1 and the antigenic prostate specific membrane antigen has a sequence within SEQ ID NO:35.

105. The isolated nucleic acid of claim 103 wherein the consecutive nucleotides are in SEQ ID NO:1 and the antigenic prostate specific membrane antigen has a sequence within SEQ ID NO:36.

106. The isolated nucleic acid of claim 103 wherein the consecutive nucleotides are in SEQ ID NO:1 and the antigenic prostate specific membrane antigen has a sequence within SEQ ID NO:37.

107. The isolated nucleic acid of claim 103 wherein the

Applicants: Ron. S. Israeli et al.
U.S. Serial No.: 09/724,026
Filed: November 28, 2000

consecutive nucleotides are in SEQ ID NO:1 and the
antigenic prostate specific membrane antigen has a sequence
within SEQ ID NO:38.

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 09/990,595
Filed: November 21, 2001

Claims 90-107

90. A hybridoma cell line selected from the group consisting of 3F5.4G6 having ATCC accession number HB12060, 3D7-1.I having ATCC accession number HB12309, 4E10 1.14 having ATCC accession number HB12310, 3E11 (ATCC HB12488), 4D8 (ATCC HB12487), 3E6 (ATCC HB12486), 3C9 (ATCC HB12484), 2C7 (ATCC HB 12490) 1G3, (ATCC HB 12489) 3C4 (ATCC HB 12494), 3C6, (ATCC HB12491), 4D4 (ATCC HB12493), 1G9 (ATCC HB12495) 5C8B9 (ATCC HB12492) and 3G6 (ATCC HB12485).
91. A monoclonal antibody having an antigen-binding region specific for the extracellular domain of prostate specific membrane antigen, said domain comprising the amino acid sequence from residue #44 to 750 as depicted in FIG.1 (SEQ ID NO:2), the antigen-binding region of which competitively inhibits the immunospecific binding of a second monoclonal antibody to its target epitope, which in said second antibody is produced by a hybridoma selected from the group consisting of 3F5.4G6 (ATCC HB12060), 1G3 (ATCC HB12489), and 4C8B9 (ATCC HB 12492).
92. A kit for diagnosis, prognosis, or monitoring prostate cancer, comprising the monoclonal antibody according to claim 91 or an antigen binding fragment thereof.
93. The kit according to claim 92 in which the antibody or antigen binding fragment thereof is packaged in an aqueous medium or in lyophilized form.

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 09/990,595
Filed: November 21, 2001

94. A monoclonal antibody having an antigen-binding region of an antibody produced by a hybridoma selected from the group consisting of 3F5.4G6 (ATCC HB12060) 1G3 (ATCC HB12489), and 4C8B9 (ATCC HB 12492).
95. The monoclonal antibody of claim 94, which is produced by a hybridoma selected from the group consisting of 3F5.4G6 (ATCC HB12060), 1G3 (ATCC HB12489), and 4C8B9 (ATCC HB 12492).
96. A monoclonal antibody having an antigen-binding region specific for the extracellular domain of prostatic specific membrane antigen, said domain comprising the amino acid sequence from residue #44 to 750 as depicted in FIG.1 (SEQ ID NO:2), the antigen-binding region of which competitively inhibits the immunospecific binding of a second monoclonal antibody to its target epitope, wherein said second antibody is produced by a hybridoma selected from the group consisting of 3D7-1.1 (ATCC HB12309), 4E10-1.14 (ATCC HB12310), 3C9 (ATCC HB12484) and 2C7 (ATCC HB12490).
97. A kit for diagnosis, prognosis or monitoring prostate cancer, comprising the monoclonal antibody according to claim 96 or an antigen binding fragment thereof.
98. A kit according to claim 97 in which the antibody or antigen binding fragment thereof is packaged in an aqueous medium or in lyophilized form.
99. A monoclonal antibody having an antigen-binding region of an antibody produced by a hybridoma selected from the group

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 09/990,595
Filed: November 21, 2001

consisting of 3D7-1.1, (ATCC HB12309), 4E10-1.14 (ATCC HB12310), 3C9 (ATCC HB12484) and 2C7 (ATCC HB12490).

100. The monoclonal antibody of claim 99, which is produced by a hybridoma selected from the group consisting of 3D7-1.1, (ATCC HB12309), 4E10-1.14 (ATCC HB12310), 3C9 (ATCC HB12484) and 2C7 (ATCC HB12490).
101. A monoclonal antibody having an antigen-binding region specific for the extracellular domain of prostate specific membrane antigen, said domain comprising the amino acid sequence from residue #44 to 750 as depicted in FIG.1 (SEQ ID NO:2), the antigen-binding region of which competitively inhibits the immunospecific binding of a second monoclonal antibody to its target epitope, wherein said second antibody is produced by a hybridoma selected from the group consisting of 3C6 (ATCC HB12491), 4D4 (ATCC HB 12493), and 1G9 (ATCC HB12495).
102. A kit for diagnosis, prognosis, or monitoring prostatic cancer, comprising the monoclonal antibody according to claim 101, or an antigen binding fragment thereof.
103. The kit according to claim 102 in which the antibody or antigen binding fragment thereof is packaged in an aqueous medium or in lyophilized form.
104. A monoclonal antibody having an antigen-binding region of an antibody produced by a hybridoma selected from the group consisting of 3C6 (ATCC HB12491), 4D4 (ATCC HB 12493), and 1G9

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(AT HB12495).

105. The monoclonal antibody of claim 104, which is produced by a hybridoma selected from the group consisting of 3C6 (ATCC HB12491), 4D4 (ATCC HB 12493), and 1G9 (AT HB12495).
106. A monoclonal antibody having an antigen-binding region specific for the extracellular domain of prostatic specific membrane antigen.
107. A monoclonal antibody having an antigen-binding region specific for the outer membrane domain of prostatic specific membrane antigen.

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Pending Claims 62-70 and 76

62. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the sequence Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35) and additional consecutive amino acids, provided that the sequence of the polypeptide is included within SEQ ID NO:2.
63. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the sequence Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36).
64. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the sequence Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37).
65. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the sequence Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO:38).

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66. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the following sequences:
- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35);
 - (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36); and
 - (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37).
67. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has within its structure consecutive amino acids having the following sequences:
- (a) Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID NO: 35);
 - (b) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO: 36);
 - (c) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO: 37); and
 - (d) Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO:38).
68. A purified antigenic prostate specific membrane antigen polypeptide having within its structure consecutive amino acids having the sequence set forth in SEQ ID NO: 2.
69. A purified antigenic prostate specific membrane antigen polypeptide consisting of consecutive amino acids, the sequence of which is within the sequence of the outside region of prostate specific membrane antigen, the sequence of which antigen is set forth in SEQ ID NO:2, provided that if the polypeptide comprises consecutive amino acids having

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the sequence set forth in SEQ ID NO:35, the polypeptide further comprises additional amino acids.

70. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has a sequence which is the same as a sequence within the outside region of prostate specific membrane antigen, the sequence of which antigen is set forth in SEQ ID NO:2, and the sequence of which polypeptide comprises, within its structure, consecutive amino acids having a sequence selected from the group consisting of:

- (a) Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID NO:36);
- (b) Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID NO:37); and
- (c) Ala-Gly-Ala-Leu-Val-Leu-Ala-Gly-Gly-Phe-Phe-Leu-Leu-Gly-Phe-Leu-Phe (SEQ ID NO:38)

provided that if the polypeptide comprises consecutive amino acids having the sequence set forth in SEQ ID NO:35, the polypeptide further comprises additional amino acids.

76. A purified prostate specific membrane antigen polypeptide comprising an antigenic fragment of prostate specific membrane antigen, which fragment has a sequence which is the same as a sequence within the outside region of prostate specific membrane antigen, the sequence of which antigen is set forth in SEQ ID NO:2, wherein the antigenic fragment generates as an immunogen antibodies specific to PSM antigen, provided that if the polypeptide comprises

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consecutive amino acids having the sequence set forth in
SEQ ID NO:35, the polypeptide further comprises additional
amino acids.

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 10/012,169
Filed: October 24, 2001

Pending Claims 24-29

24. A method of killing cancerous cells comprising: providing a monoclonal antibody which, when contacted with an extracellular domain of prostate specific membrane antigen, binds to the extracellular domain of prostate specific membrane antigen; and contacting vascular endothelial cells proximate to the cancerous cells with the monoclonal antibody under conditions effective to permit both binding of the monoclonal antibody to the vascular endothelial cells proximate to the cancerous cells and killing the cancerous cells; said monoclonal antibody being bound to a drug effective to kill the cancerous cells upon binding of the monoclonal antibody to said vascular endothelial cells proximate to the cancerous cells.
25. The method of claim 24, wherein the cancerous cells are prostate cancerous cells.
26. The method of claim 24, wherein the cancerous cells are kidney cells or colon cells.
27. The method of claim 24, wherein said contacting is carried out in a subject and comprises administering the monoclonal antibody to the subject under conditions effective to permit both binding of the monoclonal antibody to vascular endothelial cells proximate to the cancerous cells and killing the cancerous cells.

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28. The method of claim 27, wherein the subject is a human.

29. The method of claim 27, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously or intramuscularly.

Applicants: Ron S. Israeli et al.
U.S. Serial No.: 10/443,694
Filed: May 21, 2003

Pending Claims 24-27

24. A method comprising contacting a cell of neovasculature of a subject with a ligand comprising an antibody that binds to an extracellular domain of PSMA having the sequence of SEQ ID NO. 2 under conditions permitting the formation of a complex so as to form a complex between the antibody and a cell of neovasculature of a subject with cancer.
25. The method of claim 24 wherein the cell comprises endothelial cells of the neovasculature
26. The method of claim 24 wherein the antibody is a monoclonal antibody.
27. The method of claim 25 wherein the antibody is a monoclonal antibody.

*Application
for
United States Letters Patent*

To all whom it may concern:

Be it known that

Ron S. Israeli, Warren D. W. Heston, William R. Fair, Ouathek
Ouerfelli and John Pinto

have invented certain new and useful improvements in

PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

of which the following is a full, clear and exact description.

PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

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This invention disclosed herein was made in part with Government support under Grants Nos. DK47650 and
15 CA58192, CA-39203, CA-29502, CA-08748-29 from the National Institute of Health, U.S. Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

20 BACKGROUND OF THE INVENTION

Throughout this application various references are referred to within parentheses. Disclosures of these publications in their entirety are hereby
25 incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of each set of Examples in the Experimental Details section.

30

Prostate cancer is among the most significant medical problems in the United States, as the disease is now the most common malignancy diagnosed in American males. In 1992 there were over 132,000 new cases of prostate
35 cancer detected with over 36,000 deaths attributable to the disease, representing a 17.3% increase over 4 years (8). Five year survival rates for patients with prostate cancer range from 88% for those with localized disease to 29% for those with metastatic disease. The

rapid increase in the number of cases appears to result in part from an increase in disease awareness as well as the widespread use of clinical markers such as the secreted proteins prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) (7).

The prostate gland is a site of significant pathology affected by conditions such as benign growth (BPH), neoplasia (prostatic cancer) and infection (prostatitis). Prostate cancer represents the second leading cause of death from cancer in man (7). However prostatic cancer is the leading site for cancer development in men. The difference between these two facts relates to prostatic cancer occurring with increasing frequency as men age, especially in the ages beyond 60 at a time when death from other factors often intervenes. Also, the spectrum of biologic aggressiveness of prostatic cancer is great, so that in some men following detection the tumor remains a latent histologic tumor and does not become clinically significant, whereas in other it progresses rapidly, metastasizes and kills the man in a relatively short 2-5 year period (7 and 59).

In prostate cancer cells, two specific proteins that are made in very high concentrations are prostatic acid phosphatase (PAP) and prostate specific antigen (PSA) (21, 47, and 65). These proteins have been characterized and have been used to follow response to therapy. With the development of cancer, the normal architecture of the gland becomes altered, including loss of the normal duct structure for the removal of secretions and thus the secretions reach the serum. Indeed measurement of serum PSA is suggested as a potential screening method for prostatic cancer. Indeed, the relative amount of PSA and/or PAP in the cancer reduces as compared to normal or benign tissue.

PAP was one of the earliest serum markers for detecting metastatic spread (47). PAP hydrolyses tyrosine phosphate and has a broad substrate specificity. Tyrosine phosphorylation is often increased with oncogenic transformation. It has been hypothesized that during neoplastic transformation there is less phosphatase activity available to inactivate proteins that are activated by phosphorylation on tyrosine residues. In some instances, insertion of phosphatases that have tyrosine phosphatase activity has reversed the malignant phenotype.

PSA is a protease and it is not readily appreciated how loss of its activity correlates with cancer development (21, and 65). The proteolytic activity of PSA is inhibited by zinc. Zinc concentrations are high in the normal prostate and reduced in prostatic cancer. Possibly the loss of zinc allows for increased proteolytic activity by PSA. As proteases are involved in metastasis and some proteases stimulate mitotic activity, the potentially increased activity of PSA could be hypothesized to play a role in the tumors metastases and spread (39).

Both PSA and PAP are found in prostatic secretions. Both appear to be dependent on the presence of androgens for their production and are substantially reduced following androgen deprivation.

Prostate-specific membrane antigen (PSM) which appears to be localized to the prostatic membrane has been identified. This antigen was identified as the result of generating monoclonal antibodies to a prostatic cancer cell, LNCaP (22).

Dr. Horoszewicz established a cell line designated LNCaP from the lymph node of a hormone refractory,

heavily pretreated patient (23). This line was found to have an aneuploid human male karyotype. It maintained prostatic differentiation functionality in that it produced both PSA and PAP. It possessed an
5 androgen receptor of high affinity and specificity. Mice were immunized with LNCaP cells and hybridomas were derived from sensitized animals. A monoclonal antibody was derived and was designated 7E11-C5 (22). The antibody staining was consistent with a membrane
10 location and isolated fractions of LNCaP cell membranes exhibited a strongly positive reaction with immunoblotting and ELISA techniques. This antibody did not inhibit or enhance the growth of LNCaP cells in vitro or in vivo. The antibody to this antigen was
15 remarkably specific to prostatic epithelial cells, as no reactivity was observed in any other component. Immunohistochemical staining of cancerous epithelial cells was more intense than that of normal or benign epithelial cells.

20 Dr. Horoszewicz also reported detection of immunoreactive material using 7E11-C5 in serum of prostatic cancer patients (22). The immunoreactivity was detectable in nearly 60% of patients with stage D-2
25 disease and in a slightly lower percentage of patients with earlier stage disease, but the numbers of patients in the latter group are small. Patients with benign prostatic hyperplasia (BPH) were negative. Patients with no apparent disease were negative, but 50-60% of
30 patients in remission yet with active stable disease or with progression demonstrated positive serum reactivity. Patients with non prostatic tumors did not show immunoreactivity with 7E11-C5.

35 The 7E11-C5 monoclonal antibody is currently in clinical trials. The aldehyde groups of the antibody were oxidized and the linker-chelator glycol-tyrosyl-

(n, ε-diethylenetriamine-pentacetic acid)-lysine (GYK-DTPA) was coupled to the reactive aldehydes of the heavy chain. The resulting antibody was designated CYT-356. Immunohistochemical staining patterns were similar except that the CYT-356 modified antibody stained skeletal muscle. The comparison of CYT-356 with 7E11-C5 monoclonal antibody suggested both had binding to type 2 muscle fibers. The reason for the discrepancy with the earlier study, which reported skeletal muscle to be negative, was suggested to be due to differences in tissue fixation techniques. Still, the most intense and definite reaction was observed with prostatic epithelial cells, especially cancerous cells. Reactivity with mouse skeletal muscle was detected with immunohistochemistry but not in imaging studies. The Indium¹¹¹-labeled antibody localized to LNCaP tumors grown in nude mice with an uptake of nearly 30% of the injected dose per gram tumor at four days. In-vivo, no selective retention of the antibody was observed in antigen negative tumors such as PC-3 and DU-145, or by skeletal muscle. Very little was known about the PSM antigen. An effort at purification and characterization has been described at meetings by Dr. George Wright and colleagues (14 and 64).

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C:

5 Immunohistochemical detection of PSM
antigen expression in prostate cell
lines. Top panel reveals uniformly
high level of expression in LNCaP
cells; middle panel and lower panel are
10 DU-145 and PC-3 cells respectively,
both negative.

Figure 2: Autoradiogram of protein gel revealing
products of PSM coupled in-vitro
transcription/translation. Non-
15 glycosylated PSM polypeptide is seen at
84 kDa (lane 1) and PSM glycoprotein
synthesized following the addition of
microsomes is seen at 100 kDa (lane 2).

20 Figure 3: Western Blot analysis detecting PSM
expression in transfected non-PSM
expressing PC-3 cells. 100 kDa PSM
glycoprotein species is clearly seen in
LNCaP membranes (lane 1), LNCaP crude
25 lysate (lane 2), and PSM-transfected
PC-3 cells (lane 4), but is
undetectable in native PC-3 cells (lane
3).

30 Figure 4: Autoradiogram of ribonuclease
protection gel assaying for PSM mRNA
expression in normal human tissues.
Radiolabeled 1 kb DNA ladder (Gibco-
BRL) is shown in lane 1. Undigested
35 probe is 400 nucleotides (lane 2),
expected protected PSM band is 350
nucleotides, and tRNA control is shown

5 (lane 3). A strong signal is seen in
human prostate (lane 11), with very
faint, but detectable signals seen in
human brain (lane 4) and human salivary
gland (lane 12). No signal was detected
in lane 5 kidney, lane 6 liver, lane 7
lung, lane 8 mammary gland, lane 9
pancreas, lane 10 placenta, lane 13
skeletal muscle, lane 14 spleen, and
10 lane 15 testes.

Figure 5: Autoradiogram of ribonuclease
protection gel assaying for PSM mRNA
expression in LNCaP tumors grown in
15 nude mice, and in human prostatic
tissues. ³²P-labeled 1 kb DNA ladder is
shown in lane 1. 298 nucleotide
undigested probe is shown (lane 2), and
tRNA control is shown (lane 3). PSM
20 mRNA expression is clearly detectable
in LNCaP cells (lane 4), orthotopically
grown LNCaP tumors in nude mice with
and without matrigel (lanes 5 and 6),
and subcutaneously implanted and grown
25 LNCaP tumors in nude mice (lane 7).
PSM mRNA expression is also seen in
normal human prostate (lane 8), and in
a moderately differentiated human
prostatic adenocarcinoma (lane 10).
30 Very faint expression is seen in a
sample of human prostate tissue with
benign hyperplasia (lane 9).

Figure 6: Ribonuclease protection assay for PSM
35 expression in LNCaP cells treated with
physiologic doses of various steroids
for 24 hours. ³²P-labeled DNA ladder is

5 shown in lane 1. 298 nucleotide
undigested probe is shown (lane 2), and
tRNA control is shown (lane 3). PSM
mRNA expression is highest in untreated
LNCaP cells in charcoal-stripped media
(lane 4). Applicant see significantly
diminished PSM expression in LNCaP
cells treated with DHT (lane 5),
10 Testosterone (lane 6), Estradiol (lane
7), and Progesterone (lane 8), with
little response to Dexamethasone (lane
9).

15 **Figure 7:** Data illustrating results of PSM DNA
and RNA presence in transfect Dunning
cell lines employing Southern and
Northern blotting techniques

Figures 8A-8B:
20 Figure A indicates the power of
cytokine transfected cells to teach
unmodified cells. Administration was
directed to the parental flank or
prostate cells. The results indicate
25 the microenvironment considerations.

Figure B indicates actual potency at a
particular site. The tumor was
implanted in prostate cells and treated
30 with immune cells at two different
sites.

Figures 9A-9B:
35 Relates potency of cytokines in
inhibiting growth of primary tumors.
Animals administered un-modified
parental tumor cells and administered



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Figures 15A-15D:

- 5 DNA sequence containing promoter elements from nucleotide -1 to nucleotide -3017. -1 is upstream of start site of PSM.
- Figure 16: Potential binding sites on the PSM promoter fragment.
- 10 Figure 17: Promoter activity of PSM up-stream fragment/CAT gene chimera.
- 15 Figure 18: Comparison between PSM and PSM' cDNA. Sequence of the 5' end of PSM cDNA (32) is shown. Underlined region (beginning at nucleotide 115 and continuing to nucleotide 380) denotes nucleotides which are absent in PSM' cDNA but present in PSM cDNA.. Boxed region represents the putative transmembrane domain of PSM antigen. * Asterisk denotes the putative translation initiation site for PSM'.
- 20
- 25 Figure 19: Graphical representation of PSM and PSM' cDNA sequences and antisense PSM RNA probe (b). PSM cDNA sequence with complete coding region (32). (a) PSM' cDNA sequence from this study. (c) Cross hatched and open boxes denote sequences identity in PSM and PSM'. Hatched box indicates sequence absent from PSM'. Regions of cDNA sequence complementary to the antisense probe are indicated by dashed lines between the sequences.
- 30
- 35

- Figure 20: RNase protection assay with PSM specific probe in primary prostatic tissues. Total cellular RNA was isolated from human prostatic samples: normal prostate, BPH, and CaP. PSM and PSM' spliced variants are indicated with arrows at right. The left lane is a DNA ladder. Samples from different patients are classified as: lanes 3-6, CaP, carcinoma of prostate; BPH, benign prostatic hypertrophy, lanes 7-9; normal, normal prostatic tissue, lanes 10-12. Autoradiograph was exposed for longer period to read lanes 5 and 9.
- Figure 21: Tumor Index, a quantification of the expression of PSM and PSM'. Expression of PSM and PSM' was quantified by densitometry and expressed as a ratio of PSM/PSM' on the Y-axis. Three samples each were quantitated for primary CaP, BPH and normal prostate tissues. Two samples were quantitated for LNCaP. Normal, normal prostate tissue.
- Figure 22: Characterization of PSM membrane bound and PSM' in the cytosol.
- Figure 23: Photograph of ethidium bromide stained gel depicting representative negative and positive controls used in the study. Samples 1-5 were from, respectively: male with prostatitis, a healthy female volunteer, a male with BPH, a control 1:1,000,000 dilution of LNCaP cells, and a patient with renal

cell carcinoma. Below each reaction is the corresponding control reaction performed with beta-2-microglobulin primers to assure RNA integrity. No PCR products were detected for any of these negative controls.

Figure 24:

Photograph of gel displaying representative positive PCR results using PSM primers in selected patients with either localized or disseminated prostate cancer. Sample 1-5 were from, respectively: a patient with clinically localized stage T1_c disease, a radical prostatectomy patient with organ confined disease and a negative serum PSA, a radical prostatectomy patient with locally advanced disease and a negative serum PSA, a patient with treated stage D2 disease, and a patient with treated hormone refractory disease.

Figure 25:

Chromosomal location of PSM based on in-situ hybridization with cDNA and with genomic cosmid.

Figure 26:

Human monochromosomal somatic cell hybrid blot showing that chromosome 11 contained the PSM genetic sequence by Southern analysis. DNA panel digested with PstI restriction enzyme and probed with PSM cDNA. Lanes M and H refer to mouse and hamster DNAs. The numbers correspond to the human chromosomal DNA in that hybrid.

5 **Figure 27:** Ribonuclease protection assay using PSM radiolabeled RNA probe reveals an abundant PSM mRNA expression in AT6.1-11 clone 1, but not in AT6.1-11 clone 2, thereby mapping PSM to 11p11.2-13 region.

10 **Figure 28:** Tissue specific expression of PSM RNA by Northern blotting and RNase protection assay.

15 **Figure 29:** Mapping of the PSM gene to the 11p11.2-p13 region of human chromosome 11 by southern blotting and in-situ hybridization.

20 **Figure 30:** Schematic of potential response elements.

25 **Figure 31:** Schematic depiction of metastatic prostate cell transfected with promoter for PSM which is driving expression of prodrug activating enzyme cytosine deaminase. This allows for prostate specific expression and tumor localized conversion of non-toxic 5 fluorocytosine to 5 flurouracil.

30 **Figure 32A-32C:** Nucleic acid of PSM genomic DNA is read 5 prime away from the transcription start site: number on the sequences indicates nucleotide upstream from the start site. Therefore, nucleotide #121 is actually -121 using conventional numbering system.

35

- 5 **Figure 33:** Representation of NAAG 1, acivudin, azotomycin, and 6-diazo-5-oxo-norleucine, DON.
- 10 **Figure 34:** Representation of N-acetylaspartylglutamate (NAAG), PALA, PALAGLU, phosphonate antagonist of glutamate receptor and phosphonates of PALAGLU and NAAG.
- 15 **Figure 35:** Synthesis of N-acetylaspartylglutamate, NAAG 1.
- 20 **Figure 36:** Synthesis of N-phosphonoacetylaspartyl-L-glutamate.
- 25 **Figure 37:** Synthesis of 5-diethylphosphono-2-amino benzylvalerate intermediate.
- 30 **Figure 38:** Synthesis of analog 4 and 5.
- 35 **Figure 39:** Representation of DON, analogs 17-20.
- Figure 40:** Substrates for targeted drug delivery, analog 21 and 22.
- Figure 41:** Dynemycin A and its mode of action.
- Figure 42:** Synthesis of analog 28.
- Figure 43:** Synthesis for intermediate analog 28.
- Figure 44:** Attachment points for PALA.
- Figure 45:** Mode of action for substrate 21.

Figures 46A-46D:

Intron 1F: Forward Sequence.

Figures 47A-47E:

5 Intron 1R: Reverse Sequence

Figures 48A-48C:

Intron 2F: Forward Sequence

10 **Figures 49A-49C:**

Intron 2R: Reverse Sequence

Figures 50A-50B:

15 Intron 3F: Forward Sequence

Figures 51A-51B:

Intron 3R: Reverse Sequence

20 **Figures 52A-52C:**

Intron 4F: Forward Sequence

Figures 53A-53E:

25 Intron 4RF: Reverse Sequence

Figure 54: PSM genomic organization of the exon and 19 intron junction sequences. The exon/intron junctions are as follows:

- 30
1. Exon /intron 1 at bp 389-390;
 2. Exon /intron 2 at bp 490-491;
 3. Exon /intron 3 at bp 681-682;
 4. Exon /intron 4 at bp 784-785;
 5. Exon /intron 5 at bp 911-912;
 6. Exon /intron 6 at bp 1096-1097;

35

 7. Exon /intron 7 at bp 1190-1191;
 8. Exon /intron 8 at bp 1289- 1290;
 9. Exon /intron 9 at bp 1375-1376;

- 5
- 10
10. Exon /intron 10 at bp 1496-1497;
 11. Exon /intron 11 at bp 1579-1580;
 12. Exon /intron 12 at bp 1643-1644;
 13. Exon /intron 13 at bp 1710-1711;
 14. Exon /intron 14 at bp 1803-1804;
 15. Exon /intron 15 at bp 1894-1895;
 16. Exon /intron 16 at bp 2158-2159;
 17. Exon /intron 17 at bp 2240-2241;
 18. Exon /intron 18 at bp 2334-2335;
 19. Exon /intron 19 at bp 2644-2645.

Figures 55A-55J:

Alternatively spliced PSM (PSM') nucleic acid sequence and amino acid sequence.

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Figure 56:

PSM pteroyl (folate) hydrolase activity in LNCaP membrane preparation. Time course of MTXglu₃ hydrolysis (- ■ -) and concurrent formation of MTXglu₂ (- -), MTXglu₁ (- ▲ -), and MTX (- -), respectively. Membrane fractions were prepared as described in Methods. Reaction volume was 100 μ L containing 50 mM acetate/Triton buffer pH 4.5, 50 μ M MTXglu₃, 10 μ g/mL protein. Values are $\bar{x} \pm$ S.D. from three separate LNCaP membrane preparations.

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Figure 57:

PSM pteroyl (folate) hydrolase activity of immunoprecipitated PSM antigen. Diagram shows typical capillary electrophoretic separation patterns of MTXglu_(n) derivatives at 0, 30, 60 and 240 minute reaction times. Elution intervals for MTXglu₃, MTXglu₂, MTXglu₁, and MTX are 4.25, 3.95, 3.55, and 3.06 min, respectively. Total volume of

30

35

reaction mixture was 100 uL containing 50 uM MTXglu₂.

Figure 58:

5 Effects of pH on gamma-glutamyl
hydrolase (PSM hydrolase) activity in
LNCaP, PC-3 PSM-transfected (PC-3(+))
and PSM non-transfected (PC-3(-))
10 cells. Enzymic activity is reported as
 μ M MTXglu₂ formed/mg protein. Each
value represents the mean of 3
reactions containing 50-60 μ g/mL
protein. The following buffers were
15 used in 50 mM concentrations spanning a
pH range of 2 to 10: glycine-HCl, pH
2.2 to 3.6; acetate, pH 3.6 to 5.6; 2-
(N-morpholino)ethanesulfonic acid
(MES), pH 5.6 to 6.8;
Tris(hydroxymethyl)aminomethane (TRIS),
20 pH 7 to 8.5; and glycine-NaOH, pH 8.6
to 10.0.

Figure 59:

25 Comparison of pteroyl hydrolase
activity in membranes isolated from
LNCaP, PC-3, TSU-Pr1, and Duke-145
adenocarcinoma cell lines. Membranes
were isolated as described in Methods.
Each value represents the mean of
triplicate reactions normalized to 1
30 mg/mL protein.

Figure 60A-60C:

35 Immunohistochemical analysis of LNCaP
and PC-3 PSM-transfected and PSM-non-
transfected cells. A 2.65 kb PSM cDNA
containing a hygromycin selection
vector was cloned into non PSM-antigen
expressing PC-3 cells and maintained in

regular media supplemented with
hygromycin B. As a control, PC-3 cells
were also transfected with the pREP7
vector alone (PC-3 PSM non-transfected
cells). Cells were permeabilized in
acetone/methanol (1:1 v/v) mixture,
blocked with 5% bovine serum
albumin/Tris buffered saline (TBS) and
the 7E11-C5 monoclonal PSM antibody was
added to cells. A secondary anti-mouse
IgG₁ antibody conjugated with alkaline
phosphatase was added and PSM-positive
cell staining performed with
bromochloroindolylphenol phosphate.
Panel A demonstrates intense
immunoreactivity associated with LNCaP
cells using the monoclonal PSM
antibody; In panel B, comparable
staining occurs in PC-3 cells
transfected with PSM expression vector.
Panel C illustrates PC-3 cells
expressing pREP7 hygromycin vector
alone.

Figure 61: Comparison of pteroyl (folate)
hydrolase activity in membranes
isolated from PSM expressing PC-3 cells
and PC-3 cells expressing pREP7
hygromycin vector alone. Membranes were
isolated as described in Methods. Each
value represents the mean of triplicate
reactions normalized to 1 mg/mL
protein.

Figure 62: Representation of N'-
acetylaspartylglutamate (NAAG), folic
acid, folate-gamma-polyglutamate,

methotrexate, methotrexate-gamma-
polyglutamate, methotrexate-alpha-
monoglutamate, methotrexate-gamma-
diglutamate, methotrexate-gamma-
5 triglutamate, methotrexate-gamma-
tétraglutamate.

Figure 63A-63B:

10 Solid phase synthesis of methotrexate
alpha-polyglutamatae analogs.

Figure 64: Sequence analysis of microsatellite
instability in PSM gene.

15 **Figure 65:** PSM genomic organization.

Figure 66: Location of microsatellite in PSM gene

SUMMARY OF THE INVENTION

5 This invention provides an isolated nucleic acid molecule encoding an alternatively spliced human prostate-specific membrane antigen. This invention provides an isolated nucleic acid comprising a promoter sequence normally associated with the transcription of a gene encoding a human prostate-specific membrane antigen. This invention provides an isolated
10 polypeptide having the biological activity of an alternatively spliced prostate-specific membrane antigen.

15 This invention provides a method of detecting a nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen and a method of detecting a prostate tumor cell in a subject.

20 Lastly, this invention provides a pharmaceutical composition comprising a compound in a therapeutically effective amount and a pharmaceutically acceptable carrier and a method of making prostate cells susceptible to a cytotoxic agent.

DETAILED DESCRIPTION OF THE INVENTION

5 This invention provides an isolated nucleic acid encoding an alternatively spliced human prostate-specific membrane (PSM') antigen. As defined herein "nucleic acid encoding an alternatively spliced prostate-specific membrane (PSM') antigen" means nucleic acid encoding a prostate-specific membrane antigen which contains a deletion in the DNA sequence
10 encoding prostate specific membrane antigen between nucleotide 115 and 380. In one embodiment the isolated nucleic acid encodes the alternatively spliced human prostate-specific membrane antigen as set forth in Figure 55.

15 This invention further provides an isolated mammalian genomic DNA molecule which encodes an alternatively spliced prostate-specific membrane antigen. This invention further provides an isolated mammalian DNA molecule of an isolated mammalian nucleic acid molecule
20 encoding an alternatively spliced prostate-specific membrane antigen. This invention also provides an isolated mammalian cDNA molecule encoding a mammalian alternatively spliced prostate-specific membrane antigen. This invention provides an isolated mammalian
25 RNA molecule encoding a mammalian alternatively spliced prostate-specific membrane antigen.

30 This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of PSM' antigen, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs and cDNAs which hybridize to the DNA and cDNA of the subject invention. Hybridization
35 methods are well known to those of skill in the art.

This invention also provides a nucleic acid molecule of

at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid molecule encoding the prostate-specific membrane antigen. This molecule may either be a DNA or RNA molecule.

5

This invention provides a nucleic acid sequence of at least 15 nucleotides capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located
10 between nucleotide 115 and nucleotide 380.

The nucleic acid molecule capable of specifically
15 hybridizing with a sequence of a nucleic acid molecule encoding the prostate-specific membrane antigen can be used as a probe. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length
20 and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes PSM antigen into suitable vectors, such as plasmids or
25 bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA
30 synthesizers.

RNA probes may be generated by inserting the PSM antigen molecule downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may
35 be produced by incubating the labeled nucleotides with the linearized PSM antigen fragment where it contains an upstream promoter in the presence of the appropriate

RNA polymerase.

For example, high stringent hybridization conditions are selected at about 5° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68°C in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68°C in a 6x SSC in a 0.6x SSX solution.

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37°C for 4 hours; 3) hybridization at 37°C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for 1 minute each at room temperature at 4x at 60°C for 30 minutes each; and 6) dry and expose to film.

The DNA molecules described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

Moreover, the isolated mammalian nucleic acid molecules encoding a mammalian prostate-specific membrane antigen and the alternatively spliced PSM' are useful for the development of probes to study the tumorigenesis of prostate cancer.

The nucleic acid molecules synthesized above may be used to detect expression of a PSM' antigen by detecting the presence of mRNA coding for the PSM antigen. Total mRNA from the cell may be isolated by many procedures well known to a person of ordinary skill in the art. The hybridizing conditions of the labelled nucleic acid molecules may be determined by routine experimentation well known in the art. The presence of mRNA hybridized to the probe may be determined by gel electrophoresis or other methods known in the art. By measuring the amount of the hybrid made, the expression of the PSM and PSM' antigen by the cell can be determined. The labeling may be radioactive. For an example, one or more radioactive nucleotides can be incorporated in the nucleic acid when it is made.

In one embodiment of this invention, nucleic acids are extracted by precipitation from lysed cells and the

mRNA is isolated from the extract using an oligo-dT column which binds the poly-A tails of the mRNA molecules. The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by luminescence autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

The probes are also useful for in-situ hybridization or in order to locate tissues which express this gene, or for other hybridization assays for the presence of this gene or its mRNA in various biological tissues. The in-situ hybridization using a labelled nucleic acid molecule is well known in the art. Essentially, tissue sections are incubated with the labelled nucleic acid molecule to allow the hybridization to occur. The molecule will carry a marker for the detection because it is "labelled", the amount of the hybrid will be determined based on the detection of the amount of the marker and so will the expression of PSM antigen.

This invention further provides isolated PSM' antigen nucleic acid molecule operatively linked to a promoter of RNA transcription. The isolated PSM' antigen sequence can be linked to vector systems. Various vectors including plasmid vectors, cosmid vectors, bacteriophage vectors and other viruses are well known to ordinary skilled practitioners. This invention further provides a vector which comprises the isolated nucleic acid molecule encoding for the PSM' antigen.

As an example to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme

to create complementary ends on both molecules which base pair with each other and are then ligated together with DNA ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available and known to an ordinary skilled practitioner.

Plasmid, p55A-PSM, was deposited on August 14, 1992 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. Plasmid, p55A-PSM, was accorded ATCC Accession Number 75294.

This invention further provides a host vector system for the production of a polypeptide having the biological activity of the alternatively spliced prostate-specific membrane antigen. These vectors may be transformed into a suitable host cell to form a host cell vector system for the production of a polypeptide having the biological activity of PSM' antigen.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such

vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors
5 are useful to produce cells that express the PSM antigen.

This invention further provides an isolated DNA or cDNA molecule described hereinabove wherein the host cell is
10 selected from the group consisting of bacterial cells (such as E.coli), yeast cells, fungal cells, insect cells and animal cells. Suitable animal cells include, but are not limited to Vero cells, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells.

15 This invention provides an isolated polypeptide having the biological activity of an alternatively spliced prostate-specific membrane antigen.

20 This invention further provides a method of producing a polypeptide having the biological activity of the prostate-specific membrane antigen which comprising growing host cells of a vector system containing the PSM' antigen sequence under suitable conditions
25 permitting production of the polypeptide and recovering the polypeptide so produced.

This invention provides a mammalian cell comprising a DNA molecule encoding a mammalian PSM' antigen, such as
30 a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a mammalian PSM' antigen and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA
35 encoding the mammalian PSM' antigen as to permit expression thereof.

Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk⁺ cells, Cos cells, etc. Expression plasmids such as that described
5 supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, electroporation or DNA encoding the mammalian PSM antigen may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain
10 mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a mammalian PSM antigen.

This invention further provides ligands bound to the mammalian PSM' antigen.

15 This invention also provides a therapeutic agent comprising a ligand identified by the above-described method and a cytotoxic agent conjugated thereto. The cytotoxic agent may either be a radioisotope or a
20 toxin. Examples of radioisotopes or toxins are well known to one of ordinary skill in the art.

This invention also provides a method of imaging prostate cancer in human patients which comprises
25 administering to the patients at least one ligand identified by the above-described method, capable of binding to the cell surface of the prostate cancer cell and labelled with an imaging agent under conditions permitting formation of a complex between the ligand
30 and the cell surface PSM' antigen. This invention further provides a composition comprising an effective imaging agent of the PSM' antigen ligand and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known to one of ordinary
35 skill in the art. For an example, such a pharmaceutically acceptable carrier can be physiological saline.

Also provided by this invention is a purified mammalian PSM' antigen. As used herein, the term "purified alternatively spliced prostate-specific membrane antigen" shall mean isolated naturally-occurring prostate-specific membrane antigen or protein (purified from nature or manufactured such that the primary, secondary and tertiary conformation, and posttranslational modifications are identical to naturally-occurring material) as well as non-naturally occurring polypeptides having a primary structural conformation (i.e. continuous sequence of amino acid residues). Such polypeptides include derivatives and analogs.

This invention provides an isolated nucleic acid comprising a promoter sequence normally associated with the transcription of a gene encoding a human prostate-specific membrane antigen. In one embodiment regulatory elements are set forth in Figure 15. In another embodiment the promoter is between nucleotide -1 to -641 of Figure 15A.

This invention provides a method to identify such natural ligand or other ligand which can bind to the PSM' antigen. A method to identify the ligand comprises a) coupling the purified mammalian PSM' antigen to a solid matrix, b) incubating the coupled purified mammalian PSM' protein with the potential ligands under the conditions permitting binding of ligands and the purified PSM' antigen; c) washing the ligand and coupled purified mammalian PSM' antigen complex formed in b) to eliminate the nonspecific binding and impurities and finally d) eluting the ligand from the bound purified mammalian PSM' antigen. The techniques of coupling proteins to a solid matrix are well known in the art. Potential ligands may

either be deduced from the structure of mammalian PSM' by other empirical experiments known by ordinary skilled practitioners. The conditions for binding may also easily be determined and protocols for carrying
5 such experimentation are known to those skilled in the art. The ligand-PSM' antigen complex will be washed. Finally, the bound ligand is eluted and characterized. Standard ligands characterization techniques are well known in the art.

10 The above method may also be used to purify ligands from any biological source. For purification of natural ligands in the cell, cell lysates, serum or other biological samples will be used to incubate with
15 the mammalian PSM' antigen bound on a matrix. Specific natural ligand will then be identified and purified as above described.

20 With the protein sequence information, antigenic areas may be identified and antibodies directed against these areas may be generated and targeted to the prostate cancer for imaging the cancer or therapies.

25 This invention provides an antibody directed against the amino acid sequence of a mammalian PSM' antigen.

This invention provides a method to select specific regions on the PSM' antigen to generate antibodies. The protein sequence may be determined from the PSM'
30 DNA sequence. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic
35 regions are well known to form the part of the protein that is inserted into the lipid bilayer of the cell membrane, while hydrophilic regions are located on the

cell surface, in an aqueous environment. Usually, the hydrophilic regions will be more immunogenic than the hydrophobic regions. Therefore the hydrophilic amino acid sequences may be selected and used to generate antibodies specific to mammalian PSM antigen. For an example, hydrophilic sequences of the human PSM antigen shown in hydrophilicity plot may be easily selected. The selected peptides may be prepared using commercially available machines. As an alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen.

Polyclonal antibodies against these peptides may be produced by immunizing animals using the selected peptides. Monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Alternatively, monoclonal antibodies may be produced by in vitro techniques known to a person of ordinary skill in the art. These antibodies are useful to detect the expression of mammalian PSM antigen in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

In one embodiment, peptides Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID No.), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No.) and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No.) of human PSM antigen are selected.

This invention further provides polyclonal and monoclonal antibody(ies) against peptides Asp-Glu-Leu-Lys-Ala-Glu (SEQ ID No.), Asn-Glu-Asp-Gly-Asn-Glu (SEQ ID No.) and Lys-Ser-Pro-Asp-Glu-Gly (SEQ ID No.).

This invention provides a method of imaging prostate cancer in human patients which comprises administering to the patient the monoclonal antibody directed against the peptide of the mammalian PSM' antigen capable of binding to the cell surface of the prostate cancer cell and labeled with an imaging agent under conditions permitting formation of a complex between the monoclonal antibody and the cell surface prostate-specific membrane antigen. The imaging agent is a radioisotope such as Indium¹¹¹.

This invention further provides a prostate cancer specific imaging agent comprising the antibody directed against PSM' antigen and a radioisotope conjugated thereto.

This invention also provides a composition comprising an effective imaging amount of the antibody directed against the PSM' antigen and a pharmaceutically acceptable carrier. The methods to determine effective imaging amounts are well known to a skilled practitioner. One method is by titration using different amounts of the antibody.

In addition to the standard pharmacophores that can be added to known structures, with the PSM transfectants one can identify potential ligands from combinatorial libraries that might not have been otherwise predicted such combinatorial libraries can be synthetic, peptide, or RNA based.

This invention further provides an immunoassay for measuring the amount of the prostate-specific membrane antigen in a biological sample comprising steps of a) contacting the biological sample with at least one antibody directed against the PSM' antigen to form a complex with said antibody and the prostate-specific

membrane antigen, and b) measuring the amount of the prostate-specific membrane antigen in said biological sample by measuring the amount of said complex. One example of the biological sample is a serum sample.

5

This invention provides a method to purify mammalian prostate-specific membrane antigen comprising steps of a) coupling the antibody directed against the PSM' antigen to a solid matrix; b) incubating the coupled antibody of a) with lysate containing prostate-specific membrane antigen under the condition which the antibody and prostate membrane specific can bind; c) washing the solid matrix to eliminate impurities and d) eluting the prostate-specific membrane antigen from the coupled antibody.

15

This invention also provides a transgenic nonhuman mammal which comprises the isolated nucleic acid molecule encoding a mammalian PSM' antigen. This invention further provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a mammalian prostate-specific membrane antigen so placed as to be transcribed into antisense mRNA complementary to mRNA encoding the prostate-specific membrane antigen and which hybridizes to mRNA encoding the prostate specific antigen thereby reducing its translation.

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Animal model systems which elucidate the physiological and behavioral roles of mammalian PSM' antigen are produced by creating transgenic animals in which the expression of the PSM' antigen is either increased or decreased, or the amino acid sequence of the expressed PSM antigen is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a mammalian PSM' antigen, by

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microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these PSM' antigen sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native PSM antigen but does express, for example, an inserted mutant PSM antigen, which has replaced the native PSM antigen in the animal's genome by recombination, resulting in under expression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added PSM antigens, resulting in over expression of the PSM antigens.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as Me medium (16). DNA or cDNA encoding a mammalian PSM antigen is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected

is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse
5 stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here
10 only for exemplary purposes.

Another use of the PSM antigen sequence is to isolate homologous gene or genes in different mammals. The gene or genes can be isolated by low stringency screening of
15 either cDNA or genomic libraries of different mammals using probes from PSM sequence. The positive clones identified will be further analyzed by DNA sequencing techniques which are well known to an ordinary person skilled in the art. For example, the detection of
20 members of the protein serine kinase family by homology probing.

This invention provides a method of suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor
25 cells comprising introducing a DNA molecule encoding an alternatively spliced prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell of a subject, in a way that
30 expression of the alternatively spliced prostate specific membrane antigen is under the control of the regulatory element, thereby suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells.
35 The subject may be a mammal or more specifically a human.

In one embodiment, the DNA molecule is operatively linked to a 5' regulatory element forms part of a transfer vector which is inserted into a cell or organism. In addition the vector is capable of replication and expression of the alternatively spliced prostate specific membrane antigen. The DNA molecule can be integrated into a genome of a eukaryotic or prokaryotic cell or in a host cell containing and/or expressing an alternatively spliced prostate specific membrane antigen.

Further, the DNA molecule encoding alternatively spliced prostate specific membrane antigen may be introduced by a bacterial, viral, fungal, animal, or liposomal delivery vehicle. Other means are also available and known to an ordinary skilled practitioner.

Further, the DNA molecule encoding an alternatively spliced prostate specific membrane antigen operatively linked to a promoter or enhancer. A number of viral vectors have been described including those made from various promoters and other regulatory elements derived from virus sources. Promoters consist of short arrays of nucleic acid sequences that interact specifically with cellular proteins involved in transcription. The combination of different recognition sequences and the cellular concentration of the cognate transcription factors determines the efficiency with which a gene is transcribed in a particular cell type.

Examples of suitable promoters include a viral promoter. Viral promoters include: adenovirus promoter, an simian virus 40 (SV40) promoter, a cytomegalovirus (CMV) promoter, a mouse mammary tumor virus (MMTV) promoter, a Malony murine leukemia virus promoter, a murine sarcoma virus promoter, and a Rous

antigen, or a pro-drug activating enzyme. Other means are also available and known to an ordinary skilled practitioner.

5 In addition, this invention provides a prostate tumor cell, comprising a DNA molecule isolated from mammalian nucleic acid encoding an alternatively spliced mammalian prostate-specific membrane antigen under the control a 5' regulatory element.

10 As used herein, DNA molecules include complementary DNA (cDNA), synthetic DNA, and genomic DNA.

15 This invention provides a therapeutic vaccine for preventing human prostate tumor growth or stimulation of prostate tumor cells in a subject, comprising administering an effective amount to the prostate cell, and a pharmaceutical acceptable carrier, thereby preventing the tumor growth or stimulation of tumor
20 cells in the subject. Other means are also available and known to an ordinary skilled practitioner.

This invention provides a method of detecting hematogenous micrometastatic tumor cells of a subject, comprising (A) performing nested polymerase chain
25 reaction (PCR) on blood, bone marrow or lymph node samples of the subject using the prostate specific membrane antigen primers or alternatively spliced prostate specific antigen primers, and (B) verifying
30 micrometastases by DNA sequencing and Southern analysis, thereby detecting hematogenous micrometastatic tumor cells of the subject. The subject may be a mammal or more specifically a human.

35 The micrometastatic tumor cell may be a prostatic cancer and the DNA primers may be derived from prostate specific antigen. Further, the subject may be

sarcoma virus promoter.

Further, another suitable promoter is a heat shock promoter. Additionally, a suitable promoter is a
5 bacteriophage promoter. Examples of suitable bacteriophage promoters include but not limited to, a T7 promoter, a T3 promoter, an SP6 promoter, a lambda promoter, a baculovirus promoter.

10 Also suitable as a promoter is an animal cell promoter such as an interferon promoter, a metallothionein promoter, an immunoglobulin promoter. A fungal promoter is also a suitable promoter. Examples of
15 fungal promoters include but are not limited to, an ADC1 promoter, an ARG promoter, an ADH promoter, a CYC1 promoter, a CUP promoter, an ENO1 promoter, a GAL promoter, a PHO promoter, a PGK promoter, a GAPDH promoter, a mating type factor promoter. Further,
20 plant cell promoters and insect cell promoters are also suitable for the methods described herein.

This invention provides a method of suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor
25 cells, comprising introducing a DNA molecule encoding an alternatively spliced prostate specific membrane antigen operatively linked to a 5' regulatory element coupled with a therapeutic DNA into a tumor cell of a
30 subject, thereby suppressing or modulating metastatic ability of prostate tumor cells, prostate tumor growth or elimination of prostate tumor cells. The subject may be a mammal or more specifically a human.

Further, the therapeutic DNA which is coupled to the
35 DNA molecule encoding a prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell may code for a cytokine, viral

administered with simultaneously an effective amount of hormones, so as to increase expression of prostate specific membrane antigen. Further, growth factors or cytokine may be administered in separately or in conjunction with hormones. Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor (EGF) family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factors, B-nerve growth factor, platelet-derived growth factor, vascular endothelial growth factor, interleukin 1, IL-1 receptor antagonist, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, IL-6 soluble receptor, interleukin 7, interleukin 8, interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, angiogenin, chemokines, colony stimulating factors, granulocyte-macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, adhesion molecule, and soluble tumor necrosis factor (TNF) receptors.

This invention provides a method of abrogating the mitogenic response due to transferrin, comprising introducing a DNA molecule encoding prostate specific membrane antigen operatively linked to a 5' regulatory element into a tumor cell, the expression of which gene is directly associated with a defined pathological effect within a multicellular organism, thereby abrogating mitogen response due to transferrin. The tumor cell may be a prostate cell.

This invention provides a method of determining prostate cancer progression in a subject which comprises: a) obtaining a suitable prostate tissue sample; b) extracting RNA from the prostate tissue

sample; c) performing a RNase protection assay on the RNA thereby forming a duplex RNA-RNA hybrid; d) detecting PSM and PSM' amounts in the tissue sample; e) calculating a PSM/PSM' tumor index, thereby determining prostate cancer progression in the subject. In-situ hybridization may be performed in conjunction with the above detection method.

This invention provides a method of detecting prostate cancer in a subject which comprises: (a) obtaining from a subject a prostate tissue sample; (b) treating the tissue sample so as to separately recover nucleic acid molecules present in the prostate tissue sample; (c) contacting the resulting nucleic acid molecules with multiple pairs of single-stranded labeled oligonucleotide primers, each such pair being capable of specifically hybridizing to the tissue sample, under hybridizing conditions; (d) amplifying any nucleic acid molecules to which a pair of primers hybridizes so as to obtain a double-stranded amplification product; (e) treating any such double-stranded amplification product so as to obtain single-stranded nucleic acid molecules therefrom; (f) contacting any resulting single-stranded nucleic acid molecules with multiple single-stranded labeled oligonucleotide probes, each such probe containing the same label and being capable of specifically hybridizing with such tissue sample, under hybridizing conditions; (g) contacting any resulting hybrids with an antibody to which a marker is attached and which is capable of specifically forming a complex with the labeled-probe, when the probe is present in such a complex, under complexing conditions; and (h) detecting the presence of any resulting complexes, the presence thereof being indicative of prostate cancer in a subject.

This invention provides a method of enhancing antibody

based targeting of PSM' in prostate tissue for diagnosis or therapy of prostate cancer comprising administering to a patient b-FGF in sufficient amount to cause upregulation of PSM' expression.

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This invention provides a method of enhancing antibody based targeting of PSM' in prostate tissue for diagnosis or therapy of prostate cancer comprising administering to a patient TGF in sufficient amount to cause upregulation of PSM expression or PSM'.

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This invention provides a method of enhancing antibody based targeting of PSM' in prostate tissue for diagnosis or therapy of prostate cancer comprising administering to a patient EGF in sufficient amount to cause upregulation of PSM' expression.

15

This invention provides a method of detecting in a sample the presence of a nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen which comprises: a) obtaining a suitable sample; b) extracting RNA from the sample; c) contacting the RNA with reverse transcriptase under suitable conditions to obtain a cDNA; d) contacting the cDNA under hybridizing conditions with two oligonucleotide primers, i) the first primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 3' of nucleotide 114 of such DNA sequence, with the proviso that the 3' end of the primer does not hybridize to any sequence located 5' of nucleotide 114, and ii) the second primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 5' of nucleotide 381 of such DNA sequence, with the proviso that the 5' end of the primer does not hybridize to any

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sequence located 3' of nucleotide 381; d) amplifying
any cDNA to which the primers hybridize to so as to
obtain amplification product; e) determining the size
of the amplification product; f) comparing the size of
5 the amplification product to the size of the
amplification product known to be obtained using the
same primers with a non alternatively spliced human
prostate specific membrane antigen, wherein a smaller
amplification product is indicative of the presence of
10 the alternatively spliced prostate specific membrane
antigen, so as to thereby detect the presence of the
alternatively spliced human prostate-specific membrane
antigen in the sample.

15 In one embodiment the suitable sample may be any bodily
tissue or fluid which includes but is not limited to:
blood, bone marrow, and lymph nodes.

In one embodiment the primers are at least 14-25
20 nucleotides in length. In another embodiment the
primers are at least 15 nucleotide in length. In
another embodiment the primers are 15 nucleotides in
length. In another embodiment multiple primers are
used. Construction of primers which hybridize and
25 hybridizing conditions are known to those skilled in
the art. For example, based on Figure 18 one skilled
in the art may construct primers which hybridize to the
prostate specific membrane antigen before nucleotide
114 and after nucleotide 381.

30 Further, a method of determining the amount of the
amplification product or products (i.e. 2 or more
bands) as well as the ratio of each product is known to
those skilled in the art. For example, the amount of
35 prostate specific membrane antigen or alternatively
spliced prostate specific membrane antigen may be
determined by density, binding radiolabeled probes,

autoradiography, UV spectrography, spectrophotometer, optical scan , and phospho-imaging.

5 This invention provides a method of detecting a prostate tumor cell in a subject which comprises:
which comprises: a) obtaining a suitable sample; b) extracting RNA from the sample; c) contacting the RNA with reverse transcriptase under suitable conditions to obtain a cDNA; d) contacting the cDNA under hybridizing
10 conditions with two oligonucleotide primers, i) the first primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 3' of nucleotide 114 of such DNA sequence, with the proviso
15 that the 3' end of the primer does not hybridize to any sequence located 5' of nucleotide 114, and ii) the second primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 5' of
20 nucleotide 381 of such DNA sequence, with the proviso that the 5' end of the primer does not hybridize to any sequence located 3' of nucleotide 381; d) amplifying any cDNA to which the primers hybridize to so as to obtain amplification product; e) determining the amount
25 of the amplification product; f) comparing the amount of the amplification product to the amount of the amplification product known to be obtained using the same primers with a non alternatively spliced human prostate specific membrane antigen, wherein a greater
30 amount of the prostate specific membrane antigen is indicative of a prostate tumor cell in the subject, so as to thereby detect prostate tumor cell in the subject.

35 In PCR techniques, oligonucleotide primers complementary to the two 3' borders of the DNA of the prostate specific membrane (PSM) antigen to be

amplified are synthesized. The polymerase chain reaction is then carried out using the two primers. See *PCR Protocols: A Guide to Methods and Applications*. Hybridization of PSM antigen DNA to the above nucleic acid probes can be performed by a Southern blot under
5 stringent hybridization conditions as described herein.

Oligonucleotides for use as probes or PCR primers are chemically synthesized according to the solid phase
10 phosphoramidite triester method first described by Beaucage and Carruthers using an automated synthesizer, as described in Needham-VanDevanter. Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described
15 in Pearson, J.D. and Regnier, F.E. The sequence of the synthetic oligonucleotide can be verified using the chemical degradation method of Maxam, A.M. and Gilbert, W.

Accepted means for conducting hybridization assays are known and general overviews of the technology can be had from a review of: *Nucleic Acid Hybridization: A Practical Approach*; *Hybridization of Nucleic Acids Immobilized on Solid Supports*; *Analytical Biochemistry*
20 and Innis et al., *PCR Protocols*.
25

If PCR is used in conjunction with nucleic acid hybridization, primers are designed to target a specific portion of the nucleic acid of DNA of the PSM
30 antigen. From the information provided herein, those of skill in the art will be able to select appropriate specific primers.

It will be apparent to those of ordinary skill in the art that a convenient method for determining whether a
35 probe is specific for PSM antigen or PSM' antigen utilizes a Southern blot (or Dot blot). Briefly, to

identify a target specific probe DNA is isolated from the PSM or PSM' antigen. Test DNA is transferred to a solid (e.g., charged nylon) matrix. The probes are labelled following conventional methods. Following denaturation and/or prehybridization steps known in the art, the probe is hybridized to the immobilized DNAs under stringent conditions. Stringent hybridization conditions will depend on the probe used and can be estimated from the calculated T_m (melting temperature) of the hybridized probe (see, e.g., Sambrook for a description of calculation of the T_m). For radioactively-labeled DNA or RNA probes an example of stringent hybridization conditions is hybridization in a solution containing denatured probe and 5x SSC at 65°C for 8-24 hours followed by washes in 0.1x SSC, 0.1% SDS (sodium dodecyl sulfate) at 50-65°C. In general, the temperature and salt concentration are chosen so that the post hybridization wash occurs at a temperature that is about 5°C below the T_m of the hybrid. Thus for a particular salt concentration the temperature may be selected that is 5°C below the T_m or conversely, for a particular temperature, the salt concentration is chosen to provide a T_m for the hybrid that is 5°C warmer than the wash temperature. Following stringent hybridization and washing, a probe that hybridizes to the PSM antigen or PSM' antigen as evidenced by the presence of a signal associated with the appropriate target and the absence of a signal from the non-target nucleic acids, is identified as specific. It is further appreciated that in determining probe specificity and in utilizing the method of this invention a certain amount of background signal is typical and can easily be distinguished by one of skill from a specific signal. Two fold signal over background is acceptable.

This invention provides a therapeutic agent comprising

antibodies or ligand(s) directed against PSM' antigen and a cytotoxic agent conjugated thereto or antibodies linked enzymes which activate prodrug to kill the tumor. The cytotoxic agent may either be a
5 radioisotope or toxin.

This invention provides a compound comprising a conjugate of a cytotoxic agent and one or more amino acid residues, wherein each amino acid residue is
10 glutamate or aspartate. In one embodiment the amino acid residues alternate.

Examples of cytotoxic chemotherapeutic agents or antineoplastic agents) include, but are not limited to
15 the following: Antimetabolites: Denopterin, Edatrexate, Piritrexim, Pteropterin, Tomudex, Tremetrexate, Cladribine, Fludarabine, 6-Mercaptopurine, Thiamiprine, Thioguanine, Ancitabine, Azacitidine, 6-Azaauridine, Carmofur, Cytarabine,
20 Doxifluride, Emitefur, Enocitabine, Floxuridine, Fluorouracil, Gemcitabine, and Tegafur.

Alkaloids: Docetaxel, Etoposide, Irinotecan, Paclitaxel, Teniposide, Topotecan, Vinblastine,
25 Vincristine, and Vindesine.

Alkylating agents: Alkyl Sulfonates: Busulfan, Improsulfan, Piposulfan, Aziridines, Benzodepa, Carboquone, Meuredopa, Uredopa, Ethylenimines and
30 Methylmelamines, Altretamine, Triethylenemelamine, Triethylenephosphoramidate, Triethylenethiophosphoramidate, Chlorambucil, Chlornaphazine, Cyclophosphamide, Estramustine, Ifosfamide, Mechlorethamine, Mechlorethamine Oxide Hydrochloride, Melphalan,
35 Novembiechin, Perfosfamide, Phenesterine, Prednimustine, Trofosfamide, Uracil Mustard, Carmustine, Chlorozotocin, Fotemustine, Lomustine,

Nimustine, Ranimustine, Dacarbazine, Mannomustine,
Mitbrunitol, Mitolactol, Pipobroman, Temozolomide,
Antibiotics and Analogs: Aclacinomycins, Actinomycin,
Anthramycin, Azaserine, Bleomycins, Cactinomycin,
5 Carubicin, Carzinophilin, Chromomycins, Dactinomycin,
Caunorubicin, 6-Diazo-5-oxo-L-norleucine, Doxorubicin,
Epirubicin, Idarubicin, Menogaril, Mitomycins,
Mycophenolic Acid, Nogalamycin, Olivomycins,
Peplomycin, Pirarubicin, Plicamycin, Porfiromycin,
10 Puromycin, Streptonigrin, Streptozocin, Tubercidin,
Zinostatin, Zorubicin, and L-Asparaginase.

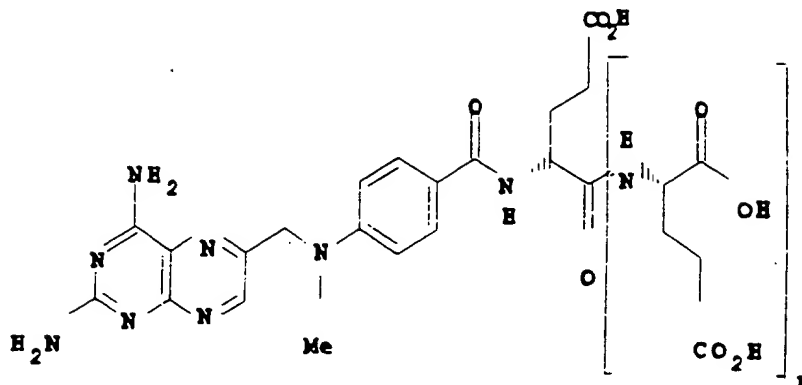
Immunomodulators: Interferon, Interferon-B, Interferon-Y,
Interleukin-2, Lentinan, Propagermanium, PSK,
15 Roquinimex, Sizofran, and Ubenimex. Platinum complexes:
Carboplatin, Cisplatin, Miboplatin, and Oxaliplatin.

Others: Aceglatone, Amsacrine, Bisantrone,
Defoosfamide, Demecolcine, Diaziquone, Eflornithine,
20 Eliptinium Acetate, Etoglucid, Fenertinide, Gallium
Nitrate, Hydroxyurea, Lonidamine, Miltefosine,
Mitoguazone, Mitoxantrone, Mopidamol, Nitracirine,
Pentostatin, Phenamet, Podophyllinic Acid 2-Ethyl-
hydrazide, Procarbazine, Razoxane, Sobuzoxane,
25 Spirogermanium, Tenuazonic Acid, Triaziquone, Urethan,
Calusterone, Dromostanolone, Epitiostanol,
Mepitiostane, Testolactone, Amiglutehimide, Mitotane,
Trilostane, Droloxifene, Tamoxifen, Toremfifene,
Aminoglutethimide, Anastrozole, Fadrozole, Formestane,
30 Letrozole, Fosfestrol, Hexestrol, Polyestradiol
Phosphate, Buserlin, Goserlin, Leuprolide, Triptorelin,
Chlormadinone Acetate, Medroxyprogesterone, Megerstrol
Acetate, Melengestrol, Porfimer Sodium, Americium,
Chromic Phosphate, Radioactive Cobalt, I-Ehtiodized
35 Oil, Gold, Radioactive, Colloidal, Iobenguane, Radium,
Radon, Sodium Iodide, Sodium Phosphate, Radioactive,
Batimastat, Folinic Acid, Amifostine, Etanidazole,

Etamidozole, and Mesna.

This invention provides a compound, wherein the compound has the structure:

5



wherein n is an integer from 1-10 inclusive.

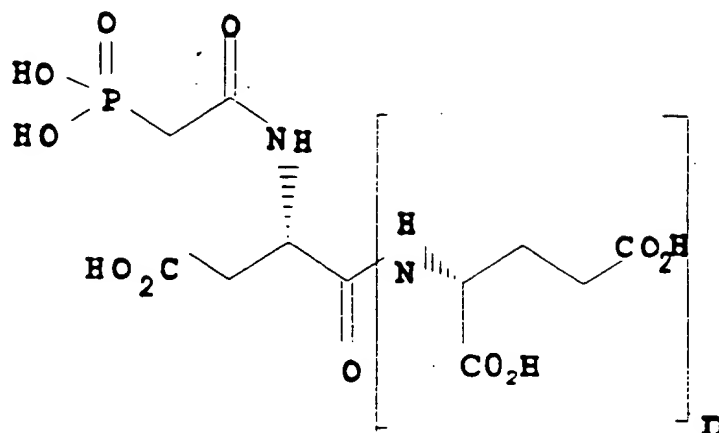
10

In one embodiment glutamate may be in L or D to form either 4-amino-N¹⁰-methyl pteroyl-L-glutamate or 4-amino-N¹⁰-methyl pteroyl-D-glutamate. In another embodiment aspartate may substitute the glutamate to form 4-amino-N¹⁰-methyl pteroyl-L-aspartate. In another embodiment aspartate may substitute the glutamate to form 4-amino-N¹⁰-methyl pteroyl-D-aspartate. In another embodiment the 4-amino-N¹⁰-methyl pteroyl may have alternating glutamate or aspartat moieties. The glutamate or aspartate are bound to the methotrexate at the alpha carbon position of methotrexate.

20

25

This invention provides a compound, wherein the compound has the structure:



5

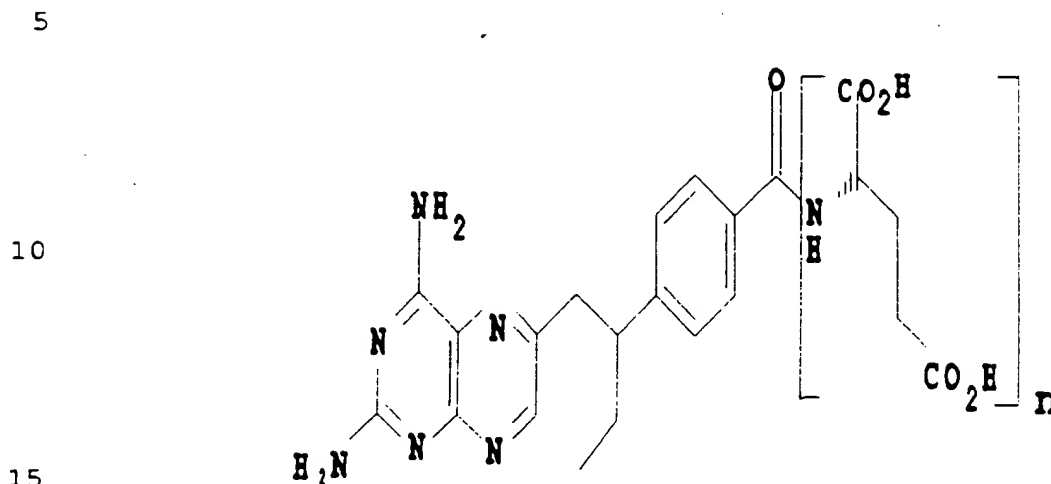
wherein n is an integer from 1-10 inclusive.

In one embodiment glutamate may be in the L or D to
 10 form either N-phosphonoacetyl-L-aspartyl (PALA)-
 glutamate or N-phosphonoacetyl-D-aspartyl-glutamate. In
 another embodiment aspartate may substitute the
 glutamate to form N-phosphonoacetyl-L-aspartyl-
 aspartate. In another embodiment the 4-amino-N¹⁰-methyl
 15 pteroyl may have alternating glutamate or aspartate
 moieties.

20

25

This invention provides a compound, wherein the compound has the structure:



wherein n is an integer from 1-10 inclusive.

20 In one embodiment glutamate may be in the L or D to form either 4-amino-10-ethyl-10-deazapteroyl (EDAM) - L-glutamate or 4-amino-10-ethyl-10-deazapteroyl-D-glutamate. In another embodiment aspartate may substitute the glutamate to form 4-amino-10-ethyl-10-deazapteroyl-L-aspartate. In another embodiment the 4-

25 amino-10-ethyl-10-deazapteroyl may have alternating glutamate or aspartat moieties.

30 This invention provides a pharmaceutical composition comprising any of the above compounds in a therapeutically effective amount and a pharmaceutically acceptable carrier.

35 This invention provides a method of making prostate cells susceptible to a cytotoxic agent, which comprises contacting the prostate cells with any of the above compounds in an amount effective to render the prostate cells susceptible to the cytotoxic chemotherapeutic

agent.

This invention provides a pharmaceutical composition comprising an effective amount the alternatively
5 spliced PSM' and a carrier or diluent. Further, this invention provides a method for administering to a subject, preferably a human, the pharmaceutical composition. Further, this invention provides a composition comprising an amount of the alternatively
10 spliced PSM' and a carrier or diluent. Specifically, this invention may be used as a food additive.

The compositions are administered in a manner compatible with the dosage formulation, and in a
15 therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each subject.

20 In one embodiment the therapeutic effective amount is 100-10,000 mg/m² IV with rescue. In another embodiment the therapeutic effective amount is 300-1000 mg/m² IV or continuous infusion. In another embodiment the therapeutic effective amount is 100 mg/m² IV continuous
25 infusion. In another embodiment the therapeutic effective amount is 40-75 mg/m² rapidly. In another embodiment the therapeutic effective amount is 30 mg/m² for 3 days by continuous IV.

30 Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration.

35

As used herein administration means a method of administering to a subject. Such methods are well

known to those skilled in the art and include, but are not limited to, administration topically, parenterally, orally, intravenously, intramuscularly, subcutaneously or by aerosol. Administration of PSM may be effected continuously or intermittently.

The pharmaceutical formulations or compositions of this invention may be in the dosage form of solid, semi-solid, or liquid such as, e.g., suspensions, aerosols or the like. Preferably the compositions are administered in unit dosage forms suitable for single administration of precise dosage amounts. The compositions may also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants; or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. Effective amounts of such diluent or carrier are those amounts which are effective to obtain a pharmaceutically acceptable formulation in terms of solubility of components, or biological activity, etc

This invention also provides a method of detecting a subject with cancer comprising a) contacting a cell of the neo-vasculature of a subject with a ligand which binds to the extracellular domain of the PSM antigen under conditions permitting formation of a complex; and b) detecting the complex with a labelled imaging

agent, thereby detecting a subject with cancer.

5 In one embodiment the cancer is, but is not limited to:
kidney, colon, or bladder. In one embodiment the
ligand is CYT-356. In another embodiment the ligand is
any antibody, monoclonal or polyclonal which binds to
the extracellular domain of PSM antigen. In one
embodiment the cells of endothelial cells of the neo-
vasculature of a subject with cancer.

10

This invention will be better understood from the
Experimental Details which follow. However, one
skilled in the art will readily appreciate that the
specific methods and results discussed are merely
15 illustrative of the invention as described more fully
in the claims which follow thereafter.

EXPERIMENTAL DETAILS

EXAMPLE 1:

5 EXPRESSION OF THE PROSTATE SPECIFIC MEMBRANE ANTIGEN

A 2.65 kb complementary DNA encoding PSM was cloned. Immunohistochemical analysis of the LNCaP, DU-145, and PC-3 prostate cancer cell lines for PSM expression
10 using the 7E11-C5.3 antibody reveals intense staining in the LNCaP cells, with no detectable expression in both the DU-145 and PC-3 cells. Coupled *in-vitro* transcription/ translation of the 2.65 kb full-length PSM cDNA yields an 84 kDa protein corresponding to the
15 predicted polypeptide molecular weight of PSM. Post-translational modification of this protein with pancreatic canine microsomes yields the expected 100 kDa PSM antigen. Following transfection of PC-3 cells with the full-length PSM cDNA in a eukaryotic
20 expression vector applicant's detect expression of the PSM glycoprotein by Western analysis using the 7E11-C5.3 monoclonal antibody. Ribonuclease protection analysis demonstrates that the expression of PSM mRNA is almost entirely prostate-specific in human tissues.
25 PSM expression appears to be highest in hormone-deprived states and is hormonally modulated by steroids, with DHT down regulating PSM expression in the human prostate cancer cell line LNCaP by 8-10 fold, testosterone down regulating PSM by 3-4 fold, and
30 corticosteroids showing no significant effect. Normal and malignant prostatic tissues consistently show high PSM expression, whereas heterogeneous, and at times absent, from expression of PSM in benign prostatic hyperplasia. LNCaP tumors implanted and grown both
35 orthotopically and subcutaneously in nude mice, abundantly express PSM providing an excellent *in-vivo* model system to study the regulation and modulation of

PSM expression.

Materials and Methods:

5 **Cells and Reagents:** The LNCaP, DU-145, and PC-3 cell
lines were obtained from the American Type Culture
Collection. Details regarding the establishment and
characteristics of these cell lines have been
previously published. Unless specified otherwise,
10 LNCaP cells were grown in RPMI 1640 media supplemented
with L-glutamine, nonessential amino acids, and 5%
fetal calf serum (Gibco-BRL, Gaithersburg, MD.) in a
CO₂ incubator at 37C. DU-145 and PC-3 cells were grown
in minimal essential medium supplemented with 10% fetal
15 calf serum. All cell media were obtained from the
MSKCC Media Preparation Facility. Restriction and
modifying enzymes were purchased from Gibco-BRL unless
otherwise specified.

20 **Immunohistochemical Detection of PSM:** Avidin-biotin
method of detection was employed to analyze prostate
cancer cell lines for PSM antigen expression. Cell
cytospins were made on glass slides using 5x10⁴
cells/100ul per slide. Slides were washed twice with
25 PBS and then incubated with the appropriate suppressor
serum for 20 minutes. The suppressor serum was drained
off and the cells were incubated with diluted 7E11-C5.3
(5g/ml) monoclonal antibody for 1 hour. Samples were
then washed with PBS and sequentially incubated with
30 secondary antibodies for 30 minutes and with avidin-
biotin complexes for 30 minutes. Diaminobenzidine
served as the chromogen and color development followed
by hematoxylin counterstaining and mounting. Duplicate
cell cytospins were used as controls for each
35 experiment. As a positive control, the anti-
cytokeratin monoclonal antibody CAM 5.2 was used
following the same procedure described above. Human EJ

bladder carcinoma cells served as a negative control.

In-Vitro Transcription/Translation of PSM Antigen:

Plasmid 55A containing the full length 2.65 kb PSM cDNA
5 in the plasmid pSPORT 1 (Gibco-BRL) was transcribed in-
vitro using the Promega TNT system (Promega Corp.
Madison, WI). T7 RNA polymerase was added to the cDNA
in a reaction mixture containing rabbit reticulocyte
lysate, an amino acid mixture lacking methionine,
10 buffer, and ³⁵S-Methionine (Amersham) and incubated at
30C for 90 minutes. Post-translational modification of
the resulting protein was accomplished by the addition
of pancreatic canine microsomes into the reaction
mixture (Promega Corp. Madison, WI.). Protein products
15 were analyzed by electrophoresis on 10% SDS-PAGE gels
which were subsequently treated with Amplify
autoradiography enhancer (Amersham, Arlington Heights,
IL.) according to the manufacturers instructions and
dried at 80C in a vacuum dryer. Gels were
20 autoradiographed overnight at -70C using Hyperfilm MP
(Amersham).

Transfection of PSM into PC-3 Cells: The full length
PSM cDNA was subcloned into the pREP7 eukaryotic
25 expression vector (Invitrogen, San Diego, CA.).
Plasmid DNA was purified from transformed DH5-alpha
bacteria (Gibco-BRL) using Qiagen maxi-prep plasmid
isolation columns (Qiagen Inc., Chatsworth, CA.).
Purified plasmid DNA (6-10g) was diluted with 900ul of
30 Optimem media (Gibco-BRL) and mixed with 30ul of
Lipofectin reagent (Gibco-BRL) which had been
previously diluted with 900l of Optimem media. This
mixture was added to T-75 flasks of 40-50% confluent
PC-3 cells in Optimem media. After 24-36 hours, cells
35 were trypsinized and split into 100mm dishes
containing RPMI 1640 media supplemented with 10% fetal
calf serum and 1 mg/ml of Hygromycin B (Calbiochem, La

Jolla, CA.). The dose of Hygromycin B used was previously determined by a time course/dose response cytotoxicity assay. Cells were maintained in this media for 2-3 weeks with changes of media and Hygromycin B every 4-5 days until discrete colonies appeared. Colonies were isolated using 6mm cloning cylinders and expanded in the same media. As a control, PC-3 cells were also transfected with the pREP7 plasmid alone. RNA was isolated from the transfected cells and PSM mRNA expression was detected by both RNase Protection analysis (described later) and by Northern analysis.

Western Blot Detection of PSM Expression: Crude protein lysates were isolated from LNCaP, PC-3, and PSM-transfected PC-3 cells as previously described. LNCaP cell membranes were also isolated according to published methods. Protein concentrations were quantitated by the Bradford method using the BioRad protein reagent kit (BioRad, Richmond, CA.). Following denaturation, 20µg of protein was electrophoresed on a 10% SDS-PAGE gel at 25 mA for 4 hours. Gels were electroblotted onto Immobilon P membranes (Millipore, Bedford, MA.) overnight at 4C. Membranes were blocked in 0.15M NaCl/0.01M Tris-HCl (TS) plus 5% BSA followed by a 1 hour incubation with 7E11-C5.3 monoclonal antibody (10µg/ml). Blots were washed 4 times with 0.15M NaCl/0.01M Tris-HCl/0.05% Triton-X 100 (TS-X) and incubated for 1 hour with rabbit anti-mouse IgG (Accurate Scientific, Westbury, N.Y.) at a concentration of 10µg/ml.

Blots were then washed 4 times with TS-X and labeled with ¹²⁵I-Protein A (Amersham, Arlington Heights, IL.) at a concentration of 1 million cpm/ml. Blots were then washed 4 times with TS-X and dried on Whatman 3MM paper, followed by overnight autoradiography at -70C

using Hyperfilm MP (Amersham).

Orthotopic and Subcutaneous LNCaP Tumor Growth in Nude Mice: LNCaP cells were harvested from sub-confluent cultures by a one minute exposure to a solution of 0.25% trypsin and 0.02% EDTA. Cells were resuspended in RPMI 1640 media with 5% fetal bovine serum, washed and diluted in either Matrigel (Collaborative Biomedical Products, Bedford, MA.) or calcium and magnesium-free Hank's balanced salt solution (HBSS). Only single cell suspensions with greater than 90% viability by trypan blue exclusion were used for in vivo injection. Male athymic Swiss (nu/nu) nude mice 4-6 weeks of age were obtained from the Memorial Sloan-Kettering Cancer Center Animal Facility. For subcutaneous tumor cell injection one million LNCaP cells resuspended in 0.2 mls. of Matrigel were injected into the hindlimb of each mouse using a disposable syringe fitted with a 28 gauge needle. For orthotopic injection, mice were first anesthetized with an intraperitoneal injection of Pentobarbital and placed in the supine position. The abdomen was cleansed with Betadine and the prostate was exposed through a midline incision. 2.5 million LNCaP tumor cells in 0.1 ml. were injected directly into either posterior lobe using a 1 ml disposable syringe and a 28 gauge needle. LNCaP cells with and without Matrigel were injected. Abdominal closure was achieved in one layer using Autoclip wound clips (Clay Adams, Parsippany, N.J.). Tumors were harvested in 6-8 weeks, confirmed histologically by faculty of the Memorial Sloan-Kettering Cancer Center Pathology Department, and frozen in liquid nitrogen for subsequent RNA isolation.

RNA Isolation: Total cellular RNA was isolated from cells and tissues by standard techniques (3 and 17) as well as by using RNazol B (Cinna/Biotechx, Houston,

TX.)). RNA concentrations and quality were assessed by UV spectroscopy on a Beckman DU 640 spectrophotometer and by gel analysis. Human tissue total RNA samples were purchased from Clontech Laboratories, Inc., Palo Alto, CA.

Ribonuclease Protection Assays: A portion of the PSM cDNA was subcloned into the plasmid vector pSPORT 1 (Gibco-BRL) and the orientation of the cDNA insert relative to the flanking T7 and SP6 RNA polymerase promoters was verified by restriction analysis. Linearization of this plasmid upstream of the PSM insert followed by transcription with SP6 RNA polymerase yields a 400 nucleotide antisense RNA probe, of which 350 nucleotides should be protected from RNase digestion by PSM RNA. This probe was used in Figure 20. Plasmid IN-20, containing a 1 kb partial PSM cDNA in the plasmid pCR II (Invitrogen) was also used for riboprobe synthesis. IN-20 linearized with Xmn I (Gibco-BRL) yields a 298 nucleotide anti-sense RNA probe when transcribed using SP6 RNA polymerase, of which 260 nucleotides should be protected from RNase digestion by PSM mRNA. This probe was used in Figures 21 and 22. Probes were synthesized using SP6 RNA polymerase (Gibco-BRL), rNTPs (Gibco-BRL), RNasin (Promega), and ³²P-rCTP (NEN, Wilmington, DE.) according to published protocols (44). Probes were purified over NENSORB 20 purification columns (NEN) and approximately 1 million cpm of purified, radiolabeled PSM probe was mixed with 10 μ of each RNA and hybridized overnight at 45C using buffers and reagents from the RPA II kit (Ambion, Austin, TX). Samples were processed as per manufacturer's instructions and analyzed on 5% polyacrilamide/7M urea denaturing gels using Seq ACRYL reagents (ISS, Natick, MA.). Gels were pre-heated to 55C and run for approximately 1-2 hours at 25 watts. Gels were then fixed for 30 minutes in 10% methanol/10%

acetic acid, dried onto Whatman 3MM paper at 80C in a BioRad vacuum dryer and autoradiographed overnight with Hyperfilm MP (Amersham). Quantitation of PSM expression was determined by using a scanning laser densitometer (LKB, Piscataway, NJ.).

Steroid Modulation Experiment: LNCaP cells (2 million) were plated onto T-75 flasks in RPMI 1640 media supplemented with 5% fetal calf serum and grown 24 hours until approximately 30-40% confluent. Flasks were then washed several times with phosphate-buffered saline and RPMI medium supplemented with 5% charcoal-extracted serum was added. Cells were then grown for another 24 hours, at which time dihydrotestosterone, testosterone, estradiol, progesterone, and dexamethasone (Steraloids Inc., Wilton, NH.) were added at a final concentration of 2 nM. Cells were grown for another 24 hours and RNA was then harvested as previously described and PSM expression analyzed by ribonuclease protection analysis.

Experimental Results

Immunohistochemical Detection of PSM: Using the 7E11-C5.3 anti-PSM monoclonal antibody, PSM expression is clearly detectable in the LNCaP prostate cancer cell line, but not in the PC-3 and DU-145 cell lines (Figures 17A-17C). All normal and malignant prostatic tissues analyzed stained positively for PSM expression.

In-Vitro Transcription/Translation of PSM Antigen: As shown in Figure 18, coupled in-vitro transcription/translation of the 2.65 kb full-length PSM cDNA yields an 84 kDa protein species in agreement with the expected protein product from the 750 amino acid PSM open reading frame. Following post-translational

modification using pancreatic canine microsomes were obtained a 100 kDa glycosylated protein species consistent with the mature, native PSM antigen.

5 **Detection of PSM Antigen in LNCaP Cell Membranes and Transfected PC-3 Cells:** PC-3 cells transfected with the full length PSM cDNA in the pREP7 expression vector were assayed for expression of SM mRNA by Northern analysis. A clone with high PSM mRNA expression was
10 selected for PSM antigen analysis by Western blotting using the 7E11-C5.3 antibody. In Figure 19, the 100 kDa PSM antigen is well expressed in LNCaP cell lysate and membrane fractions, as well as in PSM-transfected PC-3 cells but not in native PC-3 cells. This
15 detectable expression in the transfected PC-3 cells proves that the previously cloned 2.65 kb PSM cDNA encodes the antigen recognized by the 7E11-C5.3 anti-prostate monoclonal antibody.

20 **PSM mRNA Expression:** Expression of PSM mRNA in normal human tissues was analyzed using ribonuclease protection assays. Tissue expression of PSM appears predominantly within the prostate, with very low levels of expression detectable in human brain and salivary
25 gland (Figure 20). No detectable PSM mRNA expression was evident in non-prostatic human tissues when analyzed by Northern analysis. On occasion it is noted that detectable PSM expression in normal human small intestine tissue, however this mRNA expression is
30 variable depending upon the specific riboprobe used. All samples of normal human prostate and human prostatic adenocarcinoma assayed have revealed clearly detectable PSM expression, whereas generally decreased or absent expression of PSM in tissues exhibiting
35 benign hyperplasia (Figure 21). In human LNCaP tumors grown both orthotopically and subcutaneously in nude mice abundant PSM expression with or without the use of

matrigel, which is required for the growth of subcutaneously implanted LNCaP cells was detected (Figure 21). PSM mRNA expression is distinctly modulated by the presence of steroids in physiologic doses (Figure 22). DHT downregulated expression by 8-10 fold after 24 hours and testosterone diminished PSM expression by 3-4 fold. Estradiol and progesterone also downregulated PSM expression in LNCaP cells, perhaps as a result of binding to the mutated androgen receptor known to exist in the LNCaP cell. Overall, PSM expression is highest in the untreated LNCaP cells grown in steroid-depleted media, a situation that simulates the hormone-deprived (castrate) state *in-vivo*. This experiment was repeated at steroid dosages ranging from 2-200 nM and at time points from 6 hours to 7 days with similar results; maximal downregulation of PSM mRNA was seen with DHT at 24 hours at doses of 2-20 nM.

20 Experimental Discussion

Previous research has provided two valuable prostatic bio-markers, PAP and PSA, both of which have had a significant impact on the diagnosis, treatment, and management of prostate malignancies. The present work describing the preliminary characterization of the prostate-specific membrane antigen (PSM) reveals it to be a gene with many interesting features. PSM is almost entirely prostate-specific as are PAP and PSA, and as such may enable further delineation of the unique functions and behavior of the prostate. The predicted sequence of the PSM protein (30) and its presence in the LNCaP cell membrane as determined by Western blotting and immunohistochemistry, indicate that it is an integral membrane protein. Thus, PSM provides an attractive cell surface epitope for antibody-directed diagnostic imaging and cytotoxic

targeting modalities . The ability to synthesize the PSM antigen in-vitro and to produce tumor xenografts maintaining high levels of PSM expression provides us with a convenient and attractive model system to further study and characterize the regulation and modulation of PSM expression. Also, the high level of PSM expression in the LNCaP cells provides an excellent in-vitro model system. Since PSM expression is hormonally-responsive to steroids and may be highly expressed in hormone-refractory disease. The detection of PSM mRNA expression in minute quantities in brain, salivary gland, and small intestine warrants further investigation, although these tissues were negative for expression of PSM antigen by immunohistochemistry using the 7E11-C5.3 antibody. In all of these tissues, particularly small intestine, mRNA expression using a probe corresponding to a region of the PSM cDNA near the 3' end, whereas expression when using a 5' end PSM probe was not detected. These results may indicate that the PSM mRNA transcript undergoes alternative splicing in different tissues.

Applicants approach is based on prostate tissue specific promotor: enzyme or cytokine chimeras. Promotor specific activation of prodrugs such as non toxic gancyclovir which is converted to a toxic metabolite by herpes simplex thymidine kinase or the prodrug 4-(bis(2chloroethyl)amino)benzoyl-L-glutamic acid to the benzoic acid mustard alkylating agent by the pseudomonas carboxy peptidase G2 was examined. As these drugs are activated by the enzyme (chimera) specifically in the tumor the active drug is released only locally in the tumor environment, destroying the surrounding tumor cells. Promotor specific activation of cytokines such as IL-12, IL-2 or GM-CSF for activation and specific antitumor vaccination is examined. Lastly the tissue specific promotor

activation of cellular death genes may also prove to be useful in this area.

5 **Gene Therapy Chimeras:** The establishment of "chimeric
DNA" for gene therapy requires the joining of different
segments of DNA together to make a new DNA that has
characteristics of both precursor DNA species involved
in the linkage. In this proposal the two pieces being
10 linked involve different functional aspects of DNA, the
promotor region which allows for the reading of the DNA
for the formation of mRNA will provide specificity and
the DNA sequence coding for the mRNA will provide for
therapeutic functional DNA.

15 **DNA-Specified Enzyme or Cytokine mRNA:** When effective,
antitumor drugs can cause the regression of very large
amounts of tumor. The main requirements for antitumor
drug activity is the requirement to achieve both a long
20 enough time (t) and high enough concentration (c) (cxt)
of exposure of the tumor to the toxic drug to assure
sufficient cell damage for cell death to occur. The
drug also must be "active" and the toxicity for the
tumor greater than for the hosts normal cells. The
availability of the drug to the tumor depends on tumor
25 blood flow and the drugs diffusion ability. Blood flow
to the tumor does not provide for selectivity as blood
flow to many normal tissues is often as great or
greater than that to the tumor. The majority of
chemotherapeutic cytotoxic drugs are often as toxic to
30 normal tissue as to tumor tissue. Dividing cells are
often more sensitive than non-dividing normal cells,
but in many slow growing solid tumors such as prostatic
cancer this does not provide for antitumor specificity.

35 Previously a means to increase tumor specificity of
antitumor drugs was to utilize tumor associated enzymes
to activate nontoxic prodrugs to cytotoxic agents. A

problem with this approach was that most of the enzymes found in tumors were not totally specific in their activity and similar substrate active enzymes or the same enzyme at only slightly lower amounts was found in
5 other tissue and thus normal tissues were still at risk for damage.

To provide absolute specificity and unique activity, viral, bacterial and fungal enzymes which have unique
10 specificity for selected prodrugs were found which were not present in human or other animal cells. Attempts to utilize enzymes such as herpes simplex thymidine kinase, bacterial cytosine deaminase and
15 carboxypeptidase G-2 were linked to antibody targeting systems with modest success. Unfortunately, antibody targeted enzymes limit the number of enzymes available per cell. Also, most antibodies do not have a high
20 tumor target to normal tissue ratio thus normal tissues are still exposed reducing the specificity of these unique enzymes. Antibodies are large molecules that have poor diffusion properties and the addition of the enzymes molecular weight further reduces the antibodies diffusion.

25 Gene therapy could produce the best desired result if it could achieve the specific expression of a protein in the tumor and not normal tissue in order that a high local concentration of the enzyme be available for the production in the tumor environment of active drug.

30

Cytokines:

Results demonstrated that tumors such as the bladder and prostate were not immunogenic, that is the
35 administration of irradiated tumor cells to the animal prior to subsequent administration of non-irradiated tumor cells did not result in a reduction of either the number of tumor cells to produce a tumor nor did it

reduce the growth rate of the tumor. But if the tumor was transfected with a retrovirus and secreted large concentrations of cytokines such as IL-2 then this could act as an antitumor vaccine and could also reduce the growth potential of an already established and growing tumor. IL-2 was the best, GM-CSF also had activity whereas a number of other cytokines were much less active. In clinical studies just using IL-2 for immunostimulation, very large concentrations had to be given which proved to be toxic. The key to the success of the cytokine gene modified tumor cell is that the cytokine is produced at the tumor site locally and is not toxic and that it stimulates immune recognition of the tumor and allows specific and non toxic recognition and destruction of the tumor. The exact mechanisms of how IL-2 production by the tumor cell activates immune recognition is not fully understood, but one explanation is that it bypasses the need for cytokine production by helper T cells and directly stimulates tumor antigen activated cytotoxic CD8 cells. Activation of antigen presenting cells may also occur.

Tissue Promotor-Specific Chimera DNA Activation

Non-Prostatic Tumor Systems:

It has been observed in non-prostatic tumors that the use of promotor specific activation can selectively lead to tissue specific gene expression of the transfected gene. In melanoma the use of the tyrosinase promotor which codes for the enzyme responsible for melanin expression produced over a 50 fold greater expression of the promotor driven reporter gene expression in melanoma cells and not non melanoma cells. Similar specific activation was seen in the melanoma cells transfected when they were growing in mice. In that experiment no non-melanoma or melanocyte cell expressed the tyrosinase drive reporter gene

product. The research group at Wellcome Laboratories have cloned and sequenced the promoter region of the gene coding for carcinoembryonic antigen (CEA). CEA is expressed on colon and colon carcinoma cells but specifically on metastatic. A gene chimera was generated which cytosine deaminase. Cytosine deaminase which converts 5 fluorocytosine into 5 fluorouracil and observed a large increase in the ability to selectively kill CEA promoter driven colon tumor cells but not normal liver cells. In vivo they observed that bystander tumor cells which were not transfected with the cytosine deaminase gene were also killed, and that there was no toxicity to the host animal as the large tumors were regressing following treatment. Herpes simplex virus, (HSV), thymidine kinase similarly activates the prodrug gancyclovir to be toxic towards dividing cancer cells and HSV thymidine kinase has been shown to be specifically activatable by tissue specific promoters.

20
Prostatic Tumor Systems: The therapeutic key to effective cancer therapy is to achieve specificity and spare the patient toxicity. Gene therapy may provide a key part to specificity in that non-essential tissues such as the prostate and prostatic tumors produce tissue specific proteins, such as acid phosphatase (PAP), prostate specific antigen (PSA), and a gene which was cloned, prostate-specific membrane antigen (PSM). Tissues such as the prostate contain selected tissue specific transcription factors which are responsible for binding to the promoter region of the DNA of these tissue specific mRNA. The promoter for PSA has been cloned. Usually patients who are being treated for metastatic prostatic cancer have been put on androgen deprivation therapy which dramatically reduces the expression of mRNA for PSA. PSM on the other hand increases in expression with hormone

deprivation which means it would be even more intensely expressed on patients being treated with hormone therapy.

5 EXAMPLE 3:

CLONING AND CHARACTERIZATION OF THE PROSTATE SPECIFIC
MEMBRANE ANTIGEN (PSM) PROMOTER.

10

The expression and regulation of the PSM gene is complex. By immunostaining, PSM antigen was found to be expressed brilliantly in metastasized tumor, and in organ confined tumor, less so in normal prostatic tissue and more heterogenous in BPH. PSM is strongly
15 expressed in both anaplastic and hormone refractory tumors. PSM mRNA has been shown to be down regulated by androgen. Expression of PSM RNA is also modulated by a host of cytokines and growth factors. Knowledge of
20 the regulation of PSM expression should aid in such diagnostic and therapeutic strategies as immunoscintigraphic imaging of prostate cancer and prostate-specific promoter-driven gene therapy.

25 Sequencing of a 3 kb genomic DNA clone revealed that two stretches of about 300 B.P. (-260 to -600; and -1325 to -1625) have substantial homology (79-87%) to known genes. The promoter lacks a GC rich region, nor does it have a consensus TATA box. However, it
30 contains a TA-rich region from position -35 to -65.

Several consensus recognition sites for general transcription factors such as AP1, AP2, NFkB, GRE and E2-RE were identified. Chimeric constructs containing
35 fragments of the upstream region of the PSM gene fused to a promoterless chloramphenicol acetyl transferase gene were transfected into, and transiently expressed

in LNCaP, PC-3, and SW620 (a colonic cell line). With an additional SV40 enhancer, sequence from -565 to +76 exhibited promoter activity in LNCaP but not in PC-3 nor in SW620.

5

Materials and Methods

Cell Lines. LNCaP and PC-3 prostatic carcinoma cell lines (American Type Culture Collection) were cultured in RPMI and MEM respectively, supplemented with 5% fetal calf serum at 37°C and 5% CO₂. SW620, a colonic cell line.

Polymerase Chain Reaction. The reaction was performed in a 50 l volume with a final concentration of the following reagents: 16.6 mM NH₄SO₄, 67 mM Tris-HCl pH 8.8, acetylated BSA 0.2 mg/ml, 2mM MgCl₂, 250µM dNTPs, 10 mM β-mercaptoethanol, and 1 U of the 111 Taq polymerase (Boehringer Mannheim, CA). A total of 25 cycles were completed with the following profile: cycle 1, 94°C 4 min.; cycle 2 through 25, 94°C 1 min, 60°C 1 min, 72°C 1 min. The final reaction was extended for 10 min at 72°C. Aliquots of the reaction were electrophoresed on 1 % agarose gels in 1X Tris-acetate-EDTA buffer.

Cloning of PSM promoter. A bacteriophage P1 library of human fibroblast genomic DNA (Genomic Sysytems, Inc., St. Louis, MI), was screened using a PCR method of Pierce et al. Primers located at the 5' end of PSM cDNA were used: 5'-CTCAAAGGGGCCGATTTC-3' and 5'-CTCTCAATCTCACTAATGCCTC-3'. A positive clone, p683, was digested with XhoI restriction enzyme. Southern analysis of the restricted fragments using a DNA probe from the extreme 5' to the Ava-I site of PSM cDNA confirmed that a 3Kb fragment contains the 5'

regulatory sequence of the PSM gene. The 3 kb XhoI fragment was subcloned into pKSBluescript vectors and sequenced using the dideoxy method.

5 **Functional Assay of PSM Promoter.** Chloramphenicol
Acetyl Transferase, (CAT) gene plasmids were
constructed from the SmaI-HindIII fragments or
subfragments (using either restriction enzyme
10 subfragments or PCR) by insertion into promoterless
pCAT basic or pCAT-enhancer vectors (Promega). pCAT-
constructs were cotransfected with pSV β gal plasmid (5
 μ g of each plasmid) into cell lines in duplicates,
using a calcium phosphate method (Gibco-BRL,
Gaithersburg, MD). The transfected cells were
15 harvested 72 hours later and assayed (15 μ g of lysate)
for CAT activity using the LSC method and for β gal
activity (Promega). CAT activities were standardized
by comparison to that of the β gal activities.

20 **Results**

Sequence of the 5' end of the PSM gene.

The DNA sequence of the 3 kb XhoI fragment of p683
which includes 3017 bp of DNA from the RNA start site
25 was determined. (Figure 15) The sequence from the XhoI
fragment displayed a remarkable arrays of elements and
motifs which are characteristic of eukaryotic promoters
and regulatory regions found in other genes (Figure
16).

30

Functional Analysis of upstream PSM genomic elements for promoter activity.

Various pCAT-PSM promoter constructs were tested for
35 promoter activities in two prostatic cell lines:
LNCaP, PC-3 and a colonic SW620 (Figure 17). Induction
of CAT activity was neither observed in p1070-CAT which

contained a 1070 bp PSM 5' promoter fragment, nor in
p676-CAT which contained a 641 bp PSM 5' promoter
fragment. However, with an additional SV-40 enhancer,
sequence from -641 to -1 (p676-CATE) exhibited promoter
5 activity in LNCaP but not in PC-3 nor in SW620.

Therefore, a LNCaP specific promoter fragment from -641
to -1 has been isolated which can be used in PSM
promoter-driven gene therapy.

10

EXAMPLE 4:

ALTERNATIVELY SPLICED VARIANTS OF PROSTATE SPECIFIC
MEMBRANE ANTIGEN RNA: RATIO OF EXPRESSION AS A
15 POTENTIAL MEASUREMENT OF PROGRESSION

MATERIALS AND METHODS

Cell Lines. LNCaP and PC-3 prostatic carcinoma cell
20 lines were cultured in RPMI and MEM respectively,
supplemented with 5% fetal calf serum at 37°C and 5%
CO₂.

Primary tissues. Primary prostatic tissues were
25 obtained from MSKCC's in-house tumor procurement
service. Gross specimen were pathologically staged by
MSKCC's pathology service.

RNA Isolation. Total RNA was isolated by a
30 modified guanidinium thiocyanate/phenol/chloroform
method using a RNazol B kit (Tel-Test, Friendswood,
TX). RNA was stored in diethyl pyrocarbonate-treated
water at -80°C. RNA was quantified using
spectrophometric absorption at 260nm.

35

cDNA synthesis. Two different batches of normal
prostate mRNAs obtained from trauma-dead males

(Clontech, Palo Alto, CA) were denatured at 70°C for 10 min., then reverse transcribed into cDNA using random hexamers and Superscript II reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) at 50°C for 30 min.
5 followed by a 94°C incubation for 5 min.

Polymerase Chain Reaction. Oligonucleotide primers (5'-CTCAAAGGGGCGGATTTCC-3' and 5'-AGGCTACTTCACTCAAAG-3'), specific for the 5' and 3' ends of PSM cDNA were designed to span the cDNA sequence.
10 The reaction was performed in a 50 µl volume with a final concentration of the following reagents: 16.6 mM NH₄SO₄, 67 mM Tris-HCl pH 8.8, acetylated BSA 0.2 mg/ml, 2mM MgCl₂, 250µM dNTPs, 10 mM β-mercaptoethanol, and 1
15 U of rTth polymerase (Perkin Elmer, Norwalk, CT). A total of 25 cycles were completed with the following profile: cycle 1, 94°C 4 min.; cycle 2 through 25, 94°C 1 min, 60°C 1 min, 72°C 1 min. The final reaction was extended for 10 min at 72°C. Aliquots of the reaction
20 were electrophoresed on 1 % agarose gels in 1X Tris-acetate-EDTA buffer.

Cloning of PCR products. PCR products were cloned by the TA cloning method into pCRII vector using a kit
25 from Invitrogen (San Diego, CA). Ligation mixture were transformed into competent *Escherichia coli* Inv5α.

Sequencing. Sequencing was done by the dideoxy method using a sequenase kit from US Biochemical
30 (Cleveland, OH). Sequencing products were electrophoresed on a 5% polyacrylamide/7M urea gel at 52°C.

RNase Protection Assays. Full length PSM cDNA clone
35 was digested with NgoM I and NheI. A 350 b.p. fragment was isolated and subcloned into pSPORT1 vector (GIBCO-BRL, Gaithersburg, MD). The resultant plasmid, pSP350,

was linearized, and the insert was transcribed by SP6 RNA polymerase to yield antisense probe of 395 nucleotide long, of which 355 nucleotides and/or 210 nucleotides should be protected from RNase digestion by PSM RNA respectively. Total cellular RNA (20 μ g) from different tissues were hybridized to the aforementioned antisense RNA probe. Assays were performed as described. tRNA was used as negative control. RPAs for LNCaP and PC-3 were repeated.

RESULTS

RT-PCR of mRNA from normal prostatic tissue. Two independent RT-PCR of mRNA from normal prostates were performed as described in Materials and Methods. Subsequent cloning and sequencing of the PCR products revealed the presence of an alternatively spliced variant, PSM'. PSM' has a shorter cDNA (2387 nucleotides) than PSM (2653 nucleotides). The results of the sequence analysis are shown in Figure 18. The cDNAs are identical except for a 266 nucleotide region near the 5' end of PSM cDNA (nucleotide 114 to 380) that is absent in PSM' cDNA. Two independent repetitions of RT-PCR of different mRNA samples yielded identical results.

RNase Protection Assays. An RNA probe complementary to PSM RNA and spanning the 3' splice junction of PSM' RNA was used to measure relative expression of PSM and PSM' mRNAs (Figure 19). With this probe, both PSM and PSM' RNAs in LNCaP cells was detected and the predominant form was PSM. Neither PSM nor PSM' RNA was detected in PC-3 cells, in agreement with previous Northern and Western blot data. Figure 20 showed the presence of both splice variants in human primary prostatic tissues. In primary prostatic tumor, PSM is the dominant form. In contrast, normal prostate expressed more PSM' than PSM. BPH samples showed about equal

expression of both variants.

5 Tumor Index. The relative expression of PSM and PSM' (Figure 36) was quantified by densitometry and expressed as a tumor index (Figure 21). LNCaP has an index ranging from 9-11; CaP from 3-6; BPH from 0.75 to 1.6; normal prostate has values from 0.075 to 0.45.

DISCUSSION

10 Sequencing data of PCR products derived from human normal prostatic mRNA with 5' and 3' end PSM oligonucleotide primers revealed a second splice variant, PSM', in addition to the previously described PSM cDNA.

15 PSM is a 750 a.a. protein with a calculated molecular weight of 84,330. PSM was hypothesized to be a type II integral membrane protein. A classic type II membrane protein is the transferrin receptor and indeed PSM has
20 a region that has modest homology with the transferrin receptor. Analysis of the PSM amino acid sequence by either the methods of Rao and Argos or Eisenburg et. al. strongly predicted one transmembrane helix in the region from a.a.#20 to #43. Both programs found other
25 regions that could be membrane associated but were not considered likely candidates for being transmembrane regions.

30 PSM' antigen, on the other hand, is a 693 a.a. protein as deduced from its mRNA sequence with a molecular weight of 78,000. PSM' antigen lacks the first 57 amino acids present in PSM antigen (Figure 18). It is likely that PSM' antigen is cytosolic.

35 The function of PSM and PSM' are probably different. The cellular location of PSM antigen suggests that it may interact with either extra- or intra- cellular

ligand(s) or both; while that of PSM' implies that PSM' can only react with cytosolic ligand(s). Furthermore, PSM antigen has 3 potential phosphorylation sites on its cytosolic domain. These sites are absent in PSM' antigen. On the other hand, PSM' antigen has 25 potential phosphorylation sites, 10 N-myristoylation sites and 9 N-glycosylation sites. For PSM antigen, all of these potential sites would be on the extracellular surface. The modifications of these sites for these homologous proteins would be different depending on their cellular locations. Consequently, the function(s) of each form would depend on how they are modified.

The relative differences in expression of PSM and PSM' by RNase protection assays was analyzed. Results of expression of PSM and PSM' in primary prostatic tissues strongly suggested a relationship between the relative expression of these variants and the status of the cell: either normal or cancerous. While it is noted here that the sample size of the study is small (Figures 20 and 21), the consistency of the trend is evident. The samples used were gross specimens from patients. The results may have been even more dramatic if specimens that were pure in content of CaP, BPH or normal had been used. Nevertheless, in these specimens, it is clear that there is a relative increase of PSM over PSM' mRNA in the change from normal to CaP. The Tumor Index (Figure 21) could be useful in measuring the pathologic state of a given sample. It is also possible that the change in expression of PSM over PSM' may be a reason for tumor progression. A more differentiated tumor state may be restored by PSM' either by transfection or by the use of differentiation agents.

EXAMPLE 5:

ENHANCED DETECTION OF PROSTATIC HEMATOGENOUS MICRO-METASTASES WITH PSM PRIMERS AS COMPARED TO PSA PRIMERS USING A SENSITIVE NESTED REVERSE TRANSCRIPTASE-PCR ASSAY.

77 randomly selected samples were analyzed from patients with prostate cancer and reveals that PSM and PSA primers detected circulating prostate cells in 48 (62.3%) and 7 (9.1%) patients, respectively. In treated stage D disease patients, PSM primers detected cells in 16 of 24 (66.7%), while PSA primers detected cells in 6 of 24 patients (25%). In hormone-refractory prostate cancer (stage D3), 6 of 7 patients were positive with both PSA and PSM primers. All six of these patients died within 2-6 months of their assay, despite aggressive cytotoxic chemotherapy, in contrast to the single patient that tested negatively in this group and is alive 15 months after his assay, suggesting that PSA-PCR positivity may serve as a predictor of early mortality. In post-radical prostatectomy patients with negative serum PSA values, PSM primers detected metastases in 21 of 31 patients (67.7%), while PSA primers detected cells in only 1 of 33 (3.0%), indicating that micrometastatic spread may be a relatively early event in prostate cancer. The analysis of 40 individuals without known prostate cancer provides evidence that this assay is highly specific and suggests that PSM expression may predict the development of cancer in patients without clinically apparent prostate cancer. Using PSM primers, micrometastases were detected in 4 of 40 controls, two of whom had known BPH by prostate biopsy and were later found to have previously undetected prostate cancer following repeat prostate biopsy performed for a rising serum PSA value. These results

show the clinical significance of detection of hematogenous micrometastatic prostate cells using PSM primers and potential applications of this molecular assay.

5

EXAMPLE 6:

MODULATION OF PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSM)
EXPRESSION IN VITRO BY CYTOKINES AND GROWTH FACTORS.

10

The effectiveness of CYT-356 imaging is enhanced by manipulating expression of PSM. PSM mRNA expression is downregulated by steroids. This is consistent with the clinical observations that PSM is strongly expressed in both anaplastic and hormone refractory lesions. In contrast, PSA expression is decreased following hormone withdrawal. In hormone refractory disease, it is believed that tumor cells may produce both growth factors and receptors, thus establishing an autocrine loop that permits the cells to overcome normal growth constraints. Many prostate tumor epithelial cells express both TGF α and its receptor, epidermal growth factor receptor. Results indicate that the effects of TGF α and other selected growth factors and cytokines on the expression of PSM in-vitro, in the human prostatic carcinoma cell line LNCaP.

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2×10^6 LNCaP cells growing in androgen-depleted media were treated for 24 to 72 hours with EGF, TGF α , TNF β or TNF α in concentrations ranging from 0.1 ng/ml to 100 ng/ml. Total RNA was extracted from the cells and PSM mRNA expression was quantitated by Northern blot analysis and laser densitometry. Both b-FGF and TGF α yielded a dose-dependent 10-fold upregulation of PSM expression, and EGF a 5-fold upregulation, compared to untreated LNCaP. In contrast, other groups have shown a marked downregulation in PSA expression induced by

these growth factors in this same in-vitro model. $TNF\alpha$, which is cytotoxic to LNCaP cells, and $TNF\beta$ downregulated PSM expression 8-fold in androgen depleted LNCaP cells.

5 TGF α is mitogenic for aggressive prostate cancer cells. There are multiple forms of PSM and only the membrane form is found in association with tumor progression. The ability to manipulate PSM expression by treatment
10 with cytokines and growth factors may enhance the efficacy of Cytogen 356 imaging, and therapeutic targeting of prostatic metastases.

EXAMPLE 7:

15 NEOADJUVANT ANDROGEN-DEPRIVATION THERAPY (ADT) PRIOR TO RADICAL PROSTATECTOMY RESULTS IN A SIGNIFICANTLY DECREASED INCIDENCE OF RESIDUAL MICROMETASTATIC DISEASE AS DETECTED BY NESTED RT-PCT WITH PRIMERS.

20 Radical prostatectomy for clinically localized prostate cancer is considered by many the "gold standard" treatment. Advances over the past decade have served to decrease morbidity dramatically. Improvements
25 intended to assist clinicians in better staging patients preoperatively have been developed, however the incidence of extra-prostatic spread still exceeds 50%, as reported in numerous studies. A phase III prospective randomized clinical study designed to
30 compare the effects of ADT for 3 months in patients undergoing radical prostatectomy with similarly matched controls receiving surgery alone was conducted. The previously completed phase II study revealed a 10% margin positive rate in the ADT group (N=69) as
35 compared to a 33% positive rate (N=72) in the surgery alone group.

Patients who have completed the phase III study were analyzed to determine if there are any differences between the two groups with respect to residual micrometastatic disease. A positive PCR result in a post-prostatectomy patient identifies viable metastatic cells in the circulation.

Nested RT-PCR was performed with PSM primers on 12 patients from the ADT group and on 10 patients from the control group. Micrometastatic cells were detected in 9/10 patients (90%) in the control group, as compared to only 2/12 (16.7%) in the ADT group. In the ADT group, 1 of 7 patients with organ-confined disease tested positively, as compared to 3 of 3 patients in the control group. In patients with extra-prostatic disease, 1 of 5 were positive in the ADT group, as compared to 6 of 7 in the control group. These results indicate that a significantly higher number of patients may be rendered tumor-free, and potentially "cured" by the use of neoadjuvant ADT.

EXAMPLE 8:

SENSITIVE NESTED RT-PCR DETECTION OF CIRCULATION PROSTATIC TUMOR CELLS - COMPARISON OF PSM AND PSA-BASED ASSAYS

Despite the improved and expanded arsenal of modalities available to clinician today, including sensitive serum PSA assays, CT scan, transrectal ultrasonography, endorectal co.I MRI, etc., many patients are still found to have metastatic disease at the time of pelvic lymph node dissection and radical prostatectomy. A highly sensitive reverse transcription PCR assay capable of detecting occult hematogenous micrometastatic prostatic cells that would otherwise go undetected by presently available staging modalities

was developed. This assay is a modification of similar PCR assays performed in patients with prostate cancer and other malignancies. The assay employs PCR primers derived from the cDNA sequences of prostate-specific antigen⁶ and the prostate-specific membrane antigen recently cloned and sequenced.

Materials and Methods

10 **Cells and Reagents.** LNCaP and MCF-7 cells were obtained from the American Type Culture Collection (Rockville, MD.). Details regarding the establishment and characteristics of these cell. Cells grown in RPMI 1640 medium and supplemented with L-glutamine, 15 nonessential amino acids, and 5% fetal calf serum (Gibco-BRL, Gaithersburg, MD.) In a 5% CO₂ incubator at 37°C. All cell media was obtained from the MSKCC Media Preparation Facility. Routine chemical reagents were of the highest grade possible and were obtained 20 from Sigma Chemical Company (St. Louis, MO).

25 **Patient Blood Specimens.** All blood specimens used in this study were from patients seen in the outpatient offices of urologists on staff at MSKCC. Two anti-coagulated tubes per patient were obtained at the time of their regularly scheduled blood draws. Specimens were obtained with informed consent of each patient , as per a protocol approved by the MSKCC Institutional Review Board. Samples were promptly brought to the 30 laboratory for immediate processing. Seventy-seven specimens from patients with prostate cancer were randomly selected and delivered to the laboratory "blinded" along with samples from negative controls for processing. These included 24 patients with stage D 35 disease (3 with D₀, 3 with D¹, 11 with D², and 7 with D³), 31 patients who had previously undergone radical prostatectomy and had undetectable postoperative serum

Determination of PCR Sensitivity. RNA was isolated from LNCaP cells and from mixtures of LNCaP and MCF-7 cells at fixed ratios (i.e. 1:100, 1:1,000, etc.) using RNazol B. Nested PCR was then performed as described
5 below with both PSA and PSM primers in order to determine the limit of detection for the assay. LNCaP:MCF-7 (1:100,000) cDNA was diluted with distilled water to obtain concentrations of 1:1,000,000. The human breast cancer cell line MCF-7 was chosen because
10 they had previously been tested by us and shown not to express either PSM nor PSA by both immunohistochemistry and conventional and nested PCR.

Polymerase Chain Reaction. The PSA outer primer
15 sequences are nucleotides 494-513 (sense) in exon 4 and nucleotides 960-979 (anti-sense) in exon 5 of the PSA cDNA. These primers yield a 486 bp PCR product from PSA CDNA that can be distinguished from a product synthesized from possible contaminating genomic DNA.

20 PSA-494 5'-TAC CCA CTG CAT CAG GAA CA-3'

PSA-960 5'-CCT TGA AGC ACA CCA TTA CA-3'

The PSA inner upstream primer begins at nucleotide 559 and the downstream primer at nucleotide 894 to yield a 355 bp PCR product.

25 PSA-559 5'-ACA CAG GCC AGG TAT TTC AG-3'

PSA-894 5'-GTC CAG CGT CCA GCA CAC AG-3'

All primers were synthesized by the MSKCC Microchemistry Core Facility. 5µg of total RNA was reverse-transcribed into cDNA using random hexamer
30 primers (Gibco-BRL) and Superscript II reverse transcriptase (Gibco-BRL) according to the manufacturers recommendations. 1µl of this CDNA served as the starting template for the outer primer PCR reaction. The 20µl PCR mix included: 0.5U Taq polymerase (Promega) Promega reaction buffer, 1.5mM
35 MgCl₂, 200µM dNTPs, and 1.0µM of each primer. This mix was then transferred to a Perkin Elmer 9600 DNA thermal

PSA levels (18 with pT2 lesions, 11 with pT3, and 2 pT4), 2 patients with locally recurrent disease following radical prostatectomy, 4 patients who had received either external beam radiation therapy or interstitial ^{125}I implants, 10 patients with untreated clinical stage T1-T2 disease, and 6 patients with clinical stage T3 disease on anti-androgen therapy. The forty blood specimens used as negative controls were from 10 health males, 9 males with biopsy-proven BPH and elevated serum PSA levels, 7 healthy females, 4 male patients with renal cell carcinoma, 2 patients with prostatic intraepithelial neoplasia (PIN), 2 patients with transitional cell carcinoma of the bladder and a pathologically normal prostate, 1 patient with acute prostatitis, 1 patient with acute promyelocytic leukemia, 1 patient with testicular cancer, 1 female patient with renal cell carcinoma, 1 patient with lung cancer, and 1 patient with a cyst of the testicle.

Blood Sample Processing/RNA Extraction. 4 ml of whole anticoagulated venous blood was mixed with 3 ml of ice cold PBS and then carefully layered atop 8 ml of Ficoll (Pharmacia, Uppsala, Sweden) in a 14-ml polystyrene tube. Tubes were centrifuged at $200 \times g$ for 30 min. at 4°C . The buffy coat layer (approx. 1 ml.) was carefully removed and rediluted to 50 ml with ice cold PBS in a 50 ml polypropylene tube. This tube was then centrifuged at $2000 \times g$ for 30 min. at 4°C . The supernatant was carefully decanted and the pellet was allowed to drip dry. One ml of RNazol B was then added to the pellet and total RNA was isolated as per manufacturers directions (Cinna/Biotechx, Houston, TX.) RNA concentrations and purity were determined by UV spectroscopy on a Beckman DU 640 spectrophotometer and by gel analysis.

cycler and incubated for 25 cycles. The PCR profile was as follows: 94°C x 15 sec., 60°C x 15 sec., and 72°C for 45 sec. After 25 cycles, samples were placed on ice, and 1µl of this reaction mix served as the template for another 25 cycles using the inner primers. The first set of tubes were returned to the thermal cycler for 25 additional cycles. The PSM outer upstream primer sequences are nucleotides 1368-1390 and the downstream primers are nucleotides 1995-2015, yielding a 67 bp PCR product.

PSM-1368 5'-CAG ATA TGT CAT TCT GGG AGG TC-3'

PSM-2015 5'-AAC ACC ATC CCT CCT CGA ACC-3'

The PSM inner upstream primer span nucleotides 1689-1713 and the downstream primer span nucleotides 1899-1923, yielding a 234 bp PCR product.

PSM-1689 5'-CCT AAC AAA AGA GCT GAA AAG CCC-3'

PSM-1923 5'-ACT GTG ATA CAG TGG ATA GCC GCT-3'

2µl of cDNA was used as the starting DNA template in the PCR assay. The 50µl PCR mix included: 1U Taq polymerase (Boehringer Mannheim), 250µM cNTPs, 10mM β-mercaptoethanol, 2mM MgCl₂, and 5µl of a 10x buffer mix containing: 166mM NH₄SO₄, 670mM Tris pH 8.8, and 2mg/ml of acetylated BSA. PCR was carried out in a Perkin Elmer 480 DNA thermal cycler with the following parameters: 94°C x 4 minutes for 1 cycle, 94°C x 30 sec., 58°C x 1 minute, and 72°C x 1 minute for 25 cycles, followed by 72°C x 10 minutes. Samples were then iced and 2.5µl of this reaction mix was used as the template for another 25 cycles with a new reaction mix containing the inner PSM primers. cDNA quality was verified by performing control reactions using primers derived from the β-2-microglobulin gene sequence¹⁰ a ubiquitous housekeeping gene. These primers span exons 2-4 and generate a 620 bp PCR product. The sequences for these primers are:

β-2 (exon 2) 5'-AGC AGA GAA TGG AAA GTC AAA-3'

β-2 (exon 4) 5'-TGT TGA TGT TGG ATA AGA GAA-3'

The entire PSA mix and 7-10μl of each PSM reaction mix were run on 1.5-2% agarose gels, stained with ethidium bromide and photographed in an Eagle Eye Video Imaging System (Statagene, Torrey Pines, CA.). Assays were repeated at least twice to verify results.

Cloning and Sequencing of PCR Products. PCR products were cloned into the PCR II plasmid vector using the TA cloning system (Invitrogen). These plasmids were transformed into competent E. coli cells using standard methods¹¹ and plasmid DNA was isolated using Magic Minipreps (Promega) and screened by restriction analysis. Double-stranded TA clones were then sequenced by the dideoxy method using ³⁵S-cCTP (NEN) and Sequenase (U.S. Biochemical). Sequencing products were then analyzed on 6% polyacrilamide/7M urea gels, which were fixed, dried, and autoradiographed as described.

Southern Analysis. PCR products were transferred from ethidium-stained agarose gels to Nytran nylon membranes (Schleicher and Schuell) by pressure blotting with a Posi-blotter (Stratagene) according to the manufacturer's instructions. DNA was cross-linked to the membrane using a UV Stratalinker (Stratagene). Blots were pre-hybridized at 65°C for 2 hours and subsequently hybridized with denatured ³²P-labeled, random-primed cDNA probes (either PSA or PSM).^{6,7} Blots were washed twice in 1x SSC/0.5% SDS at 42°C and twice in 0.1x SSC/0.1% SDS at 50°C for 20 minutes each. Membranes were air-dried and autoradiographed for 1-3 hours at room temperature with Hyperfilm MP (Amersham).

Results

35

PSA and PSM Nested PCR Assays: The application of nested PCR increased the level of detection from an

positive PSM nested PCR result which has been unable to explain. Unfortunately, this patient never returned for follow up, and thus have not been able to obtain another blood sample to repeat this assay. Positive
5 result were obtained with both PSA and PSM primers in a 61 year old male patient with renal cell carcinoma. This patient has a normal serum PSA level and a normal digital rectal examination. Overall, if the two
10 patients were excluded in whom a positive PCR, but no other clinical test, accurately predicted the presence of prostate cancer, 36/38 (94.7%) of the negative controls were negative with PSM primers, and 39/40 (97.5%) were negative using PSA primers.

15 **Patient Samples:** In a "blinded" fashion, in which the laboratory staff were unaware of the nature of each specimen, 117 samples from 77 patients mixed randomly with 40 negative controls were assayed. The patient
20 samples represented a diverse and heterogeneous group as described earlier. Several representative patient samples are displayed in Figure 49, corresponding to positive results from patients with both localized and disseminated disease. Patients 4 and 5, both with
25 stage D prostate cancer exhibit positive results with both the outer and inner primer pairs, indicating a large circulating tumor cell burden, as compared to the other samples. Although the PSM and PSA primers yielded similar sensitivities in LNCaP dilution curves as previously shown, PSM primers detected
30 micrometastases in 62.3% of the patient samples, whereas PSA primers only detected 9.1%. In patients with documented metastatic prostate cancer (stages D₀ - D₃) receiving anti-androgen treatment, PSM primers detected micrometastases in 16/24 (66.7%), whereas PSA
35 primers detected circulating cells in only 6/24 (25%). In the study 6/7 patients with hormone-refractory prostate cancer (stage D₃) were positive. In the

average of 1:10,000 using outer primers alone, to better than 1:1,000,000. Dilution curves demonstrating this added sensitivity are shown for PSA and PSM-PCR in Figures 1 and 2 respectively. Figure 1 shows that the 486 bp product of the PSA outer primer set is clearly detectable with ethidium staining to 1:10,000 dilutions, whereas the PSA inner primer 355 bp product is clearly detectable in all dilutions shown. In Figure 2 the PSM outer primer 647 bp product is also clearly detectable in dilutions to only 1:10,000 with conventional PCR, in contrast to the PSM inner nested PCR 234 bp product which is detected in dilutions as low as 1:1,000,000. Southern blotting was performed on all controls and most of the patient samples in order to confirm specificity. Southern blots of the respective dilution curves confirmed the primer specificities but did not reveal any significantly increased sensitivity.

PCR in Negative Controls: Nested PSA and PSM PCR was performed on 40 samples from patients and volunteers as described in the methods and materials section. Figure 48 reveals results from 4 representative negative control specimens, in addition to a positive control. Each specimen in the study was also assayed with the β -2-microglobulin control, as shown in the figure, in order to verify RNA integrity. Negative results were obtained on 39 of these samples using the PSA primers, however PSM nested PCR yielded 4 positive results. Two of these "false positives" represented patients with elevated serum PSA values and an enlarged prostate who underwent a transrectal prostate biopsy revealing stromal and fibromuscular hyperplasia. In both of these patients the serum PSA level continued to rise and a repeat prostate biopsy performed at a later date revealed prostate cancer. One patient who presented to the clinic with a testicular cyst was noted to have a

study, PSA primers revealed micrometastatic cells in only 1/15 (6.7%) patients with either pT3 or pT4 (locally-advanced) prostate cancer following radical prostatectomy. PSM primers detected circulating cells in 9/15 (60%) of these patients. Interestingly, circulating cells 13/18 (72.2%) patients with pT2 (organ-confined) prostate cancer following radical prostatectomy using PSM primers was detected. None of these patient samples were positive by PSA-PCR.

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Improved and more sensitive method for the detection of minimal, occult micrometastatic disease have been reported for a number of malignancies by use of immunohistochemical methods, as well as the polymerase chain reaction. The application of PCR to detect occult hematogenous micrometastases in prostate cancer was first described by Moreno, et al. using conventional PCR with PSA-derived primers.

20

When human prostate tumors and prostate cancer cells in-vitro were studied by immunohistochemistry and mRNA analysis, PSM appeared to be highly expressed in anaplastic cells, hormone-refractory cells, and bony metastases, in contrast to PSA. If cells capable of hematogenous micrometastasis represent the more aggressive and poorly-differentiated cells, they may express a higher level of PSM per cell as compared to PSA, enhancing their detectibility by RT-PCR.

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Nested RT-PCR assays are both sensitive and specific. Results have been reliably reproduced on repeated occasions. Long term testing of both cDNA and RNA stability is presently underway. Both assays are capable of detecting one prostatic cell in at least one million non-prostatic cells of similar size. This confirms the validity of the comparison of PSM vs. PSA primers. Similar levels of PSM expression in both

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a cis-acting element in the regulation of PSM expression. A polymeric chain reaction amplifying this repeat was used to look for any gene alteration in several cell lines: LNCap, PC-3, PC-3M, DU-145 as well as in 20 paired normal and early prostatic cancers (p12-4, NO). In addition, immunohistochemistry (IHC) was used to analyze PSM expression in patient samples. By IHC, no detectable expression in DU-145, PC-3, and PC-3M was found, but all tumor expressed PSM. Further sequencing data of the microsatellite repeat confirmed no change in LNCap, and in contrast, an amplification in PC-3 and a gross deletion in DU-145. Alteration of a T segment adjacent to the microsatellite repeat was found in one tumor sample. These results suggest that there is rarely alteration in the intronic microsatellite sequence of the PSM gene in early prostate cancer. The abnormal pattern in the absence of expression suggest genetic instability in the more aggressive tumor lines such as the PC-3, PC-3M and DU-145 cells.

human prostatic cancer cells in-vivo and LNCaP cells in-vitro resulted. The specificity of the PSM-PCR assay was supported by the finding that two "negative control" patients with positive PSM-PCR results were both subsequently found to have prostate cancer. This suggests an exciting potential application for this technique for use in cancer screening. In contrast to recently published data, significant ability for PSA primers to accurately detect micrometastatic cells in patients with pathologically with pathologically organ-confined prostate cancer, despite the sensitivity of the assay failed to result. Rather a surprisingly high percentage of patients with localized prostate cancer that harbor occult circulating prostate cells following "curative" radical prostatectomy results which suggests that micrometastasis is an early event in prostate cancer.

The application of this powerful new modality to potentially stage and/or follow the response to therapy in patients with prostate cancer certainly merits further investigation. In comparison to molecular detection of occult tumor cells, present clinical modalities for the detection of prostate cancer spread appear inadequate.

Transition of prostate cancer from androgen dependent to androgen independent state is a clinically important step which may be caused or accompanied by genetic changes. Expression of prostate specific membrane antigen (PSM) is most intense in LNCaP cells, an androgen dependent prostate carcinoma cell line: and is not detectable in PC-3 nor in DU-145 cells, which are androgen independent prostate carcinoma cell lines. A microsatellite repeat of (TTTTG), (TTTG), has been found in the first intron of the PSM gene. Our hypothesis is that this Microsatellite repeat could be

EXAMPLE 9:

CHROMOSOMAL LOCALIZATION OF COSMID CLONES 194 AND 683
5 BY FLUORESCENCE IN-SITU HYBRIDIZATION:

PSM was initially mapped as being located on chromosome
11p11.2-p13 (Figures 25-27). Further information from
the cDNA in-situ hybridizations experiments
10 demonstrated as much hybridization on the q as p arms.
Much larger fragments of genomic DNA was obtained as
cosmids and two of these of about 60 kilobases each one
going 3' and the other 5' both demonstrated binding to
chromosome 11 p and q under low stringency. However
15 under higher stringency conditions only the binding at
11q14-q21 remained. This result suggests that there is
another gene on 11p that is very similar to PSM because
it is so strongly binding to nearly 120 kilobases of
genomic DNA (Figure 28).

20 Purified DNA from cosmid clones 194 and 683 was
labelled with biotin dUTP by nick translation.
Labelled probes were combined with sheared human DNA
and independently hybridized to normal metaphase
25 chromosomes derived from PHA stimulated peripheral
blood lymphocytes in a solution containing 50%
formamide, 10% dextran sulfate, and 2XSSC. Specific
hybridization signals were detected by incubating the
hybridized slides in fluorescein conjugated avidin.
30 Following signal detection the slides were
counterstained with propidium iodide and analyzed.
These first experiments resulted in the specific
labelling of a group C chromosome on both the long and
short arms. This chromosome was believed to be
35 chromosome 11 on the basis of its size and morphology.
A second set of experiments were performed in which a
chromosome 11 centromere specific probe was

cohybridized with the cosmid clones. These experiments were carried out in 60% formamide in an attempt to eliminate the cross reactive signal which was observed when low stringency hybridizations were done. These experiments resulted in the specific labelling of the centromere and the long arm of chromosome 11. Measurements of 10 specifically labelled chromosomes 11 demonstrated that the cosmid clones are located at a position which is 44% of the distance from the centromere to the telomere of chromosome arm 11q, an area that corresponds to band 14q. A total of 160 metaphase cells were examined with 153 cells exhibiting specific labelling.

Cloning of the 5' upstream and 3' downstream regions of the PSM genomic DNA. A bacteriophage P1 library of human fibroblast genomic DNA (Genomic Systems, St. Louis, MI) was screened using the PCR method of Pierce et. al. Primer pairs located at either the 5' or 3' termini of PSM cDNA were used. Positive cosmid clones were digested with restriction enzymes and confirmed by Southern analysis using probes which were constructed from either the 5' or 3' ends of PSM cDNA. Positive clone p683 contains the 5' region of PSM cDNA and about 60 kb upstream region. Clone -194 contains the 3' terminal of the PSM cDNA and about 60 kb downstream.

EXAMPLE 10: PEPTIDASE ENZYMATIC ACTIVITY

Prostate Specific Membrane Antigen has activity as a carboxypeptidase and acts on both gamma linked or alpha linked amino acids which have acidic amino acids such as glutamate in the carboxy terminus.

Prostate specific membrane antigen is found in high concentration in the seminal plasma. PSM antigen has enzymatic activity with N-acetylaspartylglutamate as a

substrate and enzymatic action results in the release of, N-acetylaspartate and glutamic acid. Because PSM action will release glutamate, and because it is well known that the seminal fluid is highly enriched in its content of glutamic acid, the action of PSM antigen of endogenous protein/peptide substrates may be responsible for generating the glutamic acid present.

It is also uncertain as to the role that seminal plasma glutamic acid plays in fertility functions. It may be that interruption of PSM antigen enzymatic activity may block the generation of glutamate and could impact on seminal plasma glutamic acid levels and its attendant fertility functions. Thus agents which inhibit PSM antigen may prove to be useful in attenuating male fertility.

EXAMPLE 11: IONOTROPIC GLUTAMATE RECEPTORS IN PROSTATE TISSUE

Prostate Specific Membrane antigen acts on N-acetylaspartylglutamic acid to release glutamate and because a homologous protein has been found in the rat brain which acts on N-acetylaspartylglutamate to free glutamate and N-acetylaspartate and because these amino acids are considered to function as neurotransmitters, the enzyme is considered to be potentially important in modulating neurotransmitter excitatory amino acid signalling as a neurocarboxypeptidase. This could be important in the prostate as well, because of the neuroendocrine nature of a subpopulation of cells in the prostate which are considered to be important synthesizing neuropeptide signaling molecules. PSM antigen from the LNCaP cell was isolated and LNCaP cells can be induced to exhibit a "neuron like" phenotype.

Excitatory neurotransmission in the central nervous system (CNS) is mediated predominantly by glutamate receptors. Two types of glutamate receptors have been identified in the human CNS: metabotropic receptors, which serve G-protein coupled second messenger signalling systems, and ionotropic receptors, which serve as ligand gated ion channels. Ionotropic glutamate channels can increase the inward flow of ions such as calcium ions. This can result in the subsequent stimulation of nitric oxide, and nitric oxide modulation of a number of signalling pathways. Nitric oxide has been found to be a major signalling mechanism involved in cell growth and death, response to inflammation, smooth muscle cell contraction etc.

Methods: Detection of glutamate receptor expression was performed using anti-gluR2/3 and anti-gluR4 polyclonal antibodies and antbiotin immunohistochemical techniques in paraffin-embedded human prostate tissues.

Results: Anti-gluR2/3 immunoreactivity was unique to prostatic stroma and was absent in the prostatic epithelial compartment. Strong anti-gluR4 immunoreactivity was observed in the basal cells of the prostate. This implied a differential location and function of glutamate receptors as defined by these antibodies.

Discussion: Distribution of glutamate receptors in the prostate has not been described. Basal cells are considered the precursor cell for the prostatic acinar and neuroendocrine cells of the prostate. Glutamate receptors may provide signalling functions in their interactions with the prostate stroma and acinar cells, and PSM may be involved in that interaction. Thus inhibition or enhancement of PSM activity could serve to modulate activity of the basal cells and prove to be

a valuable aid for controlling basal cell function in the prostate.

5 The finding of glutamate like receptors in the stroma is of interest because a large part of the prostate volume is due to stromal cells. Current observation have suggested that these stromal cells have a smooth muscle cell phenotype and thus the presence of glutamate receptors may play a role in their biologic
10 function and regulation of differentiation. A most common disease in men is the abnormal benign growth of the prostate termed benign prostatic hyperplasia, BPH.

15 In areas of BPH a decrease in the level of expression of PSM antigen was observed. If PSM antigen activity is providing an aspect of the signalling for normal stromal function then the abnormal growth seen in BPH may be a response to that decreased activity and agents to restore its function could play a role in the
20 treatment or prevention of BPH.

Altering PSM antigen function may have beneficial actions outside the prostate. In the rat CNS a protein homology to PSM antigen was discovered and provides a
25 rational to consider prostate specific membrane antigen as a neurocarboxypeptidase. Alterations in its function may occur in neurotoxic disorders such as epilepsy, or ALS, alzheimers, and multiple sclerosis.

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EXAMPLE 12: IDENTIFICATION OF A MEMBRANE-BOUND
PTEROYLPOLYGAMMAGLUTAMYL
CARBOXYPEPTIDASE (FOLATE HYDROLASE)
THAT IS EXPRESSED IN HUMAN PROSTATIC
CARCINOMA

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As described PSM functions as a carboxypeptidase to hydrolyze both alpha and gamma peptide linkages with amino acids such as glutamate in the terminal carboxy position. The proximal small intestine (duodenum-strong expression PSM) but not the distal small intestine (ileum-absent PSM) was also very rich in expression of message for prostate specific membrane antigen in RNase protection assays. PSM antigen by immunohistochemistry was observed in the brush border membranes of the duodenum. This location was consistent with a hydrolase known as folate conjugase (folate hydrolase as a carboxypeptidase, not an endopeptidase) that had been described in the older literature, with the protein having been partially purified from the human small intestine. No cloning or sequencing of this gene had been done. There is a form of folate hydrolase that is found in all cells in the lysosomes and it was recently sequenced. There is no sequence relationship between the lysosomal endopeptidase. Membrane fraction of the LNCaP cells was very rich in folate hydrolase activity. The PSM specific monoclonal could be used to immunoprecipitate the folate hydrolase activity. This result always has the possibility that the folate hydrolase activity is not the same as PSM antigen but is a coprecipitating contaminant. Therefore PSM antigen was transfected into PC-3 cells. PC-3 cells do not express PSM nor do they have membrane folate hydrolase activity. In cells transfected with PSM antigen however expression of folate hydrolase activity was observed in the membranes. Thus PSM is a novel folate hydrolase,

folate carboxypeptidase, and is active in sequentially removing the terminal gamma-linked glutamates. In the proximal small intestine it is understandable why this enzyme would be in such a place, as the majority of
5 folate available from food is polygammglutamated and this enzyme is responsible for its hydrolysis.

Materials: Methotrexate triglutamate ($4\text{-NH}_2\text{-10-CH}_3\text{-PteGlu}_4$ (MTXglu₃)), pteroylpentaglutamate (PteGlu₅), and
10 para-aminobenzoylpentaglutamate, (pABAGlu₅) were purchased from Dr. B. Schircks Laboratories (Jona, Switzerland) and samples were > 98% pure when evaluated by HPLC. N-acetyl- α -aspartylglutamate (NAAG) (40
15 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Protein A Sepharose 4 Fast Flow was purchased from Pharmacia (Piscataway, NJ). The 7E11-C5 monoclonal antibody to prostate specific membrane antigen was obtained from Cytogen Corporation,
20 Princeton, NJ. All other reagents (p-hydroxymercuribenzoate, homocysteine, dithiothreitol (DTT), reduced glutathione) were of the highest purity commercially available from Sigma Chemical Co. (St. Louis, MO).

25 Culture and growth of human prostate adenocarcinoma cells (LNCaP, PC-3, TSU-Pr1, and Duke-145): LNCaP cells were maintained in defined culture medium, RPMI-1640 medium supplemented with non-essential amino acids, 5 mM glutamine, and 5% heat-inactivated fetal
30 calf serum. Duke-145, PC-3, and TSU-Pr1 cells were grown in minimal essential medium (MEM), Ham's F-12K, and MEM, respectively, containing 5% fetal calf serum. No antibiotic was included in the media. Cells (1×10^6) were plated in T-75 tissue culture flasks
35 containing 15 mL of medium and incubated at 37 °C in a humidified atmosphere of 5% CO₂. Cell numbers were determined using a Model Z F Coulter Counter (Coulter

Electronic, Inc.). Prostate cells were harvested from plates by gentle scraping at 4 °C into phosphate buffered saline (136.9 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4 , 1.47 mM KH_2PO_4 , pH 7.34, PBS) and centrifuged at 500 X g to obtain a cell pellet. Sedimented cells were routinely rinsed twice with 15 mL volumes of PBS.

Transfection of PSM into PC-3 Prostate Cell Line: The full length 2.65 kb PSM cDNA was subcloned into a pREP7 eukaryotic expression vector (Invitrogen, San Diego, CA) as previously described. Plasmid DNA was purified from transfected DH5- α (Gibco-BRL) using a Qiagen maxi prep plasmid isolation kit (Qiagen Inc., Chatsworth, CA). Purified plasmid DNA (5 μg) was diluted with 300 μL of serum free RPMI media and mixed with 45 μL of lipofectamine (Gibco-BRL) which was previously diluted with 300 μL of serum free RPMI media to allow an DNA-liposome complex to form. The mixture was kept at room temperature for 30 minutes, then added to a 60 mm petri dish containing 60-70% confluent PC-3 cells in 2.4 mL serum free RPMI. The DNA-liposome complex containing serum free media was mixed gently to ensure uniform distribution and was then incubated for 6 h at 37 °C in a CO_2 incubator. Following incubation, the media containing liposome-DNA complex was aspirated and replaced with 6 mL of regular growth media (10% fetal bovine serum, 1% penicillin-streptomycin, 1% glutamine). After 48 hours, cells were trypsinized and split 1:3 into 60 mm dishes containing regular media supplemented with 200 $\mu\text{g}/\text{mL}$ of hygromycin B (Calbiochem, LaJolla, CA). Cells were maintained for 2 weeks with changes of media containing hygromycin B every third day until discrete colonies appeared. Colonies were isolated using a 6 mm cloning cylinder and were expanded in the same media. As a control, PC-3 cells were also transfected with the pREP7 vector alone.

Immunohistochemistry: The 7E11-C5 monoclonal antibody to prostate specific antigen was used. This antibody recognizes a portion of carbohydrate-containing peptide epitope on the amino terminal end of PSM that is located on the inner portion of the cytosolic membrane. After permeabilization of LNCaP and PC-3 transfected and non-transfected cells with a mixture of acetone and methanol (1:1 v/v) and blocking with 5% bovine serum albumin in 50 mM Tris buffered saline (TBS) pH 7.45, samples were incubated with 7E11-C5 antibody (20 µg/mL) for 1 h at room temperature. Negative controls were generated by substituting the same concentration of mouse IgG2ak for the PSM antibody. Using a secondary IgG, anti-mouse antibody conjugated with alkaline phosphatase, samples were re-incubated for 1 h, rinsed in TBS, and stained with bromochloroindolylphenol phosphate in 2-amino-2-methyl-1-propanol buffer. Cells expressing PSM demonstrate an intense blue staining.

Cell Membrane Preparation: Cell lysates were prepared by sonicating approximately 6×10^6 cells in 50 mM Tris pH 7.4 buffer (2 x 10 s pulses at 20 mWatts) in an ice-bath. Membrane fractions were obtained by centrifuging lysates at 100,000 x g for 30 mins. The supernatant fractions were saved and pelleted membranes were re-suspended by gentle trituration and re-sedimented at 100,000 x g for 30 mins through 10 mL of cold 50 mM Tris pH 7.4 buffer. Washed membrane fractions were dissolved in 50 mM Tris pH 7.4 buffer containing 0.1% Triton X-100 (Tris/Triton). Enzymatic activity and immunoprecipitation preparations were performed using this membrane preparation.

Immunoprecipitation of PSM from Membrane: Membrane pellets (-1 mg protein) solubilized in Tris/Triton buffer were incubated at 4 °C for 1 h in the presence of 7E11-C5 anti-prostate monoclonal antibody (6 µg

protein). Protein A Sepharose gel equilibrated in Tris/Triton buffer was added to the immunocomplex. This preparation was subsequently incubated for an additional hour at 4 °C. Sepharose beads were
5 centrifuged at 500 x g for 5 mins and rinsed twice with Tris/Triton buffer at pH 7.4. Isolated beads were resuspended in 0.1 M glycine buffer pH 3.0, vortexed, and the supernatant fraction was assayed for hydrolase activity using MTXglu₃.

10 Pteroyl Gamma-Glutamyl Hydrolase Assay: Hydrolase activity was determined using capillary electrophoresis. The standard assay mixture contained 50 uM MTXGlu₃, 50 mM acetate buffer (pH 4.5) and enzyme
15 to a final volume of 100 uL. A sample preparation without enzyme was incubated concurrently with enzymatic assays and reactions were conducted for times varying between 0 and 240 min at 37 °C. Activities were also determined in standard reaction mixture at
20 varied pHs for 60 min. Reactions were terminated in a boiling water bath for 5 min and samples were stored frozen (-20 °C) until analysis. Following centrifugation (7,000 x g) to remove precipitated debris, capillary separation of MTX glutamated
25 analogues was performed with a Spectra Phoresis 1000 instrument (Thermo Separation, San Jose, CA) with a 75 µm id x 50 cm silica capillary (Polymicro Technology, Phoenix, AZ). Separation of pteroyl(glutamate)_n derivatives is achieved with an electrolyte of 20 mM
30 sodium borate with 15 mM sodium dodecylsulfate (pH 9.5) with +20 Kev at 25 °C. Samples were applied hydrodynamically for 1-2 s and absorbance monitored at 300 nm. Data were recorded with an IBM computer using CE-1000 software (Thermo Separation).

35 Protein determination: Protein concentrations of isolated membrane or supernatant fractions were

determined by incubating diluted aliquots with BCA reagent (Pierce Chemical Co., Rockford, IL) at 37 °C for 30 min. The spectrophotometric quantitation of protein was conducted by determining the absorbance at 562 nm against bovine serum albumin standard.

Statistical Analysis: Data were analyzed by using the Statgraphics version 4.0 program (Statistical graphics Corporation, Rockville, MD) and where summarized are expressed as mean \pm S.D. Student's unpaired t test was used to determine significance of differences.

Results:

Membrane fractions isolated from human prostate adenocarcinoma cells (LNCaP) were incubated using primarily MTXglu₃ as substrate. The time course of hydrolysis of the gamma-linked triglutamate derivative and the subsequent appearance of MTXglu₂, MTXglu₁, and MTX after 30, 60, 120, and 240 min of incubation are illustrated in Figure 82. The semipurified PSM antigen exhibits pteroyl poly gamma-glutamyl exopeptidase activity that progressively liberates all of the possible glutamates from MTXGlu₃ with accumulation of MTX.

The PSM antigen was immunoprecipitated in the presence of 7E11-C5 anti-prostate monoclonal antibody and the PSM antigen-antibody complex was adsorbed onto a Protein A Sepharose Gel column. Following twice washing of the sepharose beads with 2 mL volumes of buffer and re-solubilization of the antigen-antibody complex by adjusting the elution pH to 3.0, the supernatant fraction was assayed for hydrolase activity. Figure 55 shows the capillary electrophoretic separation of successively cleaved glutamyl moieties from MTXglu₃ after 0, 30, 60 and 240

min incubations. Results similar to these in Figure 82 were obtained using pteglu₁, with formation of folate (pteglu₁).

5 The optimum pH activity profiles of the immunoprecipitated PSM hydrolase from LNCaP cells and of the membrane fractions from PC-3 PSM-transfected and non-transfected (vector alone) cells are shown in Figure 57. The reaction was monitored as a function of
10 pH from 2 to 10 after an 1 h incubation with MTXglu₂. The extent of reaction was expressed as the concentration of MTXglu₂ formed per mg protein. Although all reaction products were detectable as illustrated in Figure 56, MTXglu₂ was the predominant
15 hydrolyzed species at incubation times ranging from 10 to 60 min. The pH profile of membrane fractions isolated from both LNCaP and PC-3 PSM-transfected cells are identical and exhibit two maxima of PSM hydrolase activity at pH 5 and 8 with no measurable activity
20 above pH 10.

To determine whether non-PSM expressing human adenocarcinoma cell lines (PC-3, TSU-Pr1, and Duke-145) exhibit folate hydrolase activity, isolated membrane
25 preparations from these cell lines were analyzed (Figure 83). The less differentiated, hormone refractory prostate cell lines (PC-3, TSU-Pr1, and Duke-145) exhibit no appreciable activity after 2 h incubations. These results are in agreement with
30 previous findings that demonstrate neither a presence of a mRNA for PSM nor antigen immunoreactivity with 7E11-C5 in these cells.

In further studies in which the cDNA for PSM was
35 transfected into non-PSM antigen expressing PC-3 cells, a close correlation between PSM antigen immunoreactivity and hydrolase activity was observed

with MTXglu₃ in membranes of LNCaP and PC-3 PSM-transfected cells (Figures 58 and 59). Immunohistochemical analyses of LNCaP (Figure 58) and PSM antigen expressing PC-3 (Figure 85B) cells revealed
5 distinct positive staining with 7E11-C5 anti-prostate monoclonal antibody. Figure 85C illustrates no immunoreactivity in PC-3 cells expressing the pREP7 hygromycin vector alone. In preparations of negative controls, all three cell lines were reacted with IgG2aK
10 rather than with 7E11-C5 antibody. No background staining resulted with the secondary antibody conjugated with alkaline phosphatase.

To compare PSM hydrolase activity with that of other
15 gamma-glutamyl hydrolases that either reside within the lysosome or are secreted as observed in several neoplastic cells, its reactivity in the presence of thiol-containing reducing agents, namely, reduced glutathione, homocysteine, and dithiothreitol (DTT),
20 and the thiol reagent, p-hydroxymercuribenzoate (PHMB), at concentrations ranging from 0.05 - 0.5 mM was observed. Of the reduced sulfhydryl derivatives, it was discovered that only DTT (≥ 0.2 mM) was slightly inhibitory ($86 \pm 3\%$ of control). Unlike gamma-linked
25 peptide hydrolase retained within the lysosome, PSM hydrolase activity was maintained in the presence of 0.5 mM PHMB.

The reactivity of PSM hydrolase against an α -glutamate dipeptide, N-acetyl- α -aspartylglutamate (NAAG), has
30 been investigated and that the PSM enzyme from either LNCaP or PSM transfected PC-3 cell membranes hydrolyses NAAG producing N-acetylaspartate and glutamate was observed. Furthermore, MTXglu₃, pteglu₃, and pABAGlu₃
35 were potent inhibitors of the PSM-mediated NAAG hydrolysis.

Discussion:

Membrane-bound PSM antigen has pteroyl poly gamma-glutamyl carboxypeptidase (folate hydrolase) activity. Gamma-glutamyl hydrolase activity is also present in lysosomes of cells and these enzymes may be responsible for regulating the length of exogenous and endogenous folyl polyglutamate chain lengths. A characteristic difference between these two hydrolases is that the PSM enzyme exhibits substantial activity at pH values 7.5 to 8.0 in addition to having an acidic pH 4.5 to 5 optimum. Moderate levels of hydrolase activity are present within LNCaP cytosolic compartment and may represent the short intracellular fragment of this class II enzyme. This reflects an interesting situation in these cells where the majority of RNA codes for the membrane-bound enzyme that is localized extracellularly. The ratio of the mRNAs in these samples that code for the class II membrane and the cytosolic proteins is ten to one. In normal prostate tissue, the mRNA coding for the membrane protein is only one-tenth that of the cytosolic form.

It is clear from this study that the prostate specific membrane antigen functions as a folate hydrolase and is unique in that it has activity on both the gamma-linked as well as the alpha linked peptide bonds. This is interesting for a number of reasons. First in the normal prostate it was demonstrated that the majority of the mRNA encodes a protein, PSM', that is likely to be cytosolic and would imply that it may be that in the prostate that folates could exist in the lesser glutamated species. If so then it means that the folate in the prostate can readily leak out and that the prostate may be subjected to "microenvironmental folate deficiencies" This may be related to the high worldwide incidence of "microscopic prostate cancer" as folate deficiencies are associated with carcinogenesis

in a number of tissues.

Benign enlargement of the prostate and prostate cancer occur in older men. It also occurs that the uptake of folate decreases with aging. If folate uptake decreases with aging this may be due to decreased PSM folate hydrolase activity in the proximal intestine. To correct such a deficiency it might be possible to use PSM folate hydrolase in foods to release the folate before consumption or take it with foods as is done with lactase in lactose intolerant individuals. If the prostate in men is susceptible to folate depletion then nutritional supplementation may help reduce the development of the microscopic lesion, indeed in some cancers such as cancer of the colon, folate supplementation was found to reduce cancer formation.

Why would the prostate cells prefer to have the lesser glutamated forms of folate? It may be that methionine synthase which is an enzyme key to folate uptake and folate utilization for one carbon methyl transfer metabolism may utilize the nonglutamated folate preferentially. In addition to folate deficiency, choline and methionine deficiency is also associated with tumor development. If shown to modulate one carbon transfers, it might be useful to inhibit this enzyme as a means to inhibit cancer development and thus serve as a chemopreventative agent. Again modulation of PSM folate hydrolase may play a role in tumor prevention and modulation of tumor growth.

A feature that cell biologists use in transfecting DNA into cells often requires selection of the transfected gene and often multiple transfections are performed. These are done with drugs that are toxic to cells such as Hygromycin and use genes that code for Hygromycin resistance which are bacterial. It may be that PSM

could be used as a selectable marker by growing the transfected cells in folate free media and including polyglutamated folate which would be able to rescue cells from folate deficiency if they expressed PSM.

5

PSM folate hydrolase activity can possibly be used as a prodrug converting enzyme. In the normal prostate PSM is intracellular. In the transformed cell the majority of the protein and its attendant enzymatic activity is extracellular in location. It may be that as the enzymes associated with cell growth require the polyglutamated forms the cancer finds a way to remove PSM folate hydrolase from the interior by alternative splicing to an extracellular enzyme. PSM is a membrane protein and is found to predominate in cancer, but PSM' is likely a cytosolic protein which predominates in the normal condition.

20 This implies that development of a prodrug that requires metabolism before it can be taken up by the tumor cell could be activated by the PSM folate hydrolase which is predominate in the cancer.

25 Methotrexate triglutamate was one of the agents used to identify the enzymatic activity of PSM antigen. Methotrexate triglutamate would not be able to use the transport protein to be taken into tumor cells, because there are specific structural requirements for folate, or methotrexate transport. If one removes the gamma-linked glutamates then methotrexate can be taken into cells and can exerts its antifolate, antitumor growth action.

35 Therefore methotrexategammatriglutamate was used to examine the action of this compound on the in vitro growth of PC-3 cells transfected with a plasmid with a

selectable marker versus a plasmid with a selectable
marker that expresses PSM antigen as well. the PC-3
cells that were transfected with PSM were inhibited 85%
in growth by day four by 10uM methotrexate
5 triglutamate, while the PC-3 plasmid only transfectants
did not exhibit any significant inhibition of growth.

PSM's folate hydrolase activity hydrolyses down to the
last glutamate which is in alpha linked position but
10 does not remove it. Because it does not remove the last
glutamate, PSM antigen's folate hydrolase activity
better serves the prodrug activation requirements of
such a prodrug. Also because it is a human enzyme it
is less likely than the carboxypeptidase G2 will cause
15 an immune response because PSM antigen is normally
present in the body.

In addition PSM could also be used as part of a prodrug
strategy that utilized gene transfer and a tissue or
20 tumor specific promoter, say such that it would be
linked to CEA promoter and PSM expressed in colon
tumors and the patients subsequently given the prodrug
such as methotrexate triglutamate. The same is also
true for the protein itself, either the whole protein
25 or the components of the active site or a modified
version that would have increased prodrug activating
activity could be linked to a delivery vehicle such as
an antibody or other specific targeting ligand,
delivered to the tumor for localization and subsequent
30 activation.

Methotrexate as a prodrug may be enhanced in
specificity by using alpha linked glutamates rather
than gamma linked glutamates because the ubiquitous
35 lysosomal hydrolase enzyme is specific for the gamma
linked bond. A pro-drug with all alpha linked
glutamates would not be a substrate, but would be a

substrate for the PSM folate hydrolase.

5 In addition to methotrexate a number of potential enzyme substrates can be employed as cytotoxic prodrugs. The synthesis of potential prodrugs, PALAglu, and a number of other potential agents are described.

10 Alpha-linked methotrexate material is synthesized by the following Merifield solid phase scheme (see Figure 88). The scheme is based on a modification of the standard Merifield solid peptide synthesis that was applied to the synthesis of methotrexate γ polyglutamates. In brief the N-Fmoc-4-terbutylglutamate
15 is first connected to the resin under standard coupling conditions using diisopropylazodicarboxylate as a coupling reagent. The Fmoc protecting group is then removed with piperidine, and this cycle would be reiterated for as many times as glutamates would be
20 needed to obtain the desired analog. For example say the pentaglutamate on solid support is the intermediate required for the preparation of methotrexate-alpha-tetraglutamate. It is deprotected at the terminal nitrogen by treatment with piperidine, then coupled
25 with pterioic acid analogue under the same conditions used above. The terbutyl and the resin are all removed in one step with 95% trifluoroacetic acid (TFA) to provide the desired material. This process is applied to every analog. The gamma linked material is provided
30 in a similar manner for use comparative studies with the alpha-linked material (see figure 89). Because of the carboxypeptidase activity a number of combination of alpha and gamma linked acidic amino acid can be optimized for their utilization of the enzyme and for
35 in vivo activity. In addition to the folate like antagonists, a number of amino acid analogs were found in the past to have antitumor activity but lacked in

vivo specificity. These agents are targetable by attaching a glutamate to the carboxy terminus of the amino acid as described and shown in the figures.

5 PALA-Glutamate 3 and analog 5, was synthesized in a similar manner with the addition to the introduction of a protected phosphonoacetate moiety instead of a simple acetate. It is compatible with the function of
10 diethylphosphonoacetic acid which allows the removal of the ethyl groups under relatively mild conditions.

Commercially available diethylphosphonoacetic acid was treated with perfluorophenyl acetate in pyridine at 0
15 deg.C to room temperature for an hour to afford the corresponding pentafluorophenyl ester in nearly quantitative yield after short path column chromatography. This was then reacted with gamma-benzylaspartate and HOAT in tetrahydrofuran for half an
20 hour at reflux temperature to give protected PALA 7 (N-phosphonoacetylaspertate) in 90% yield after flash column chromatography. The free acid was then activated as its pentafluorophenyl ester 8, then it was reacted with delta-benzyl-L-glutamate and HOAT in a mixture of THF-DMF (9:1, v/v) for 12 hours at reflux to
25 give fully protected PALA-Glutamate 9 in 66% yield after column chromatography. Sequential removal of the ethyl groups followed by the debenzylation was accomplished for a one step deprotection of both the benzyl and ethyl groups. Hence protected PALA-Glutamate was heated up to reflux in neat
30 trimethylsilylchloride for an overnight period. The resulting bistrimethylsilylphosphonate ester 10 was submitted without purification to hydrogenolysis (H_2 , 30 psi, 10% Pd/C, ethylacetate). The desired material 3
35 was isolated after purification by reverse phase column chromatography and ion exchange resin.

Analog 4 and 5 were synthesized by preparation of phosphonoglutamate 14 from the alpha-carboxyl-protected glutamate.

5 Commercially available alpha-benzyl-N-Boc-L-glutamate
11 was treated at refluxing THF with neat
boranedimethylsulfide complex to afford the
corresponding alcohol in 90% yield. This was
transformed into bromide 12 by the usual procedure
10 ($\text{PPh}_3, \text{CBr}_4$).

The Michaelis-Arbuzov reaction using triethylphosphite
to give the corresponding diethylphosphonate 13 which
would be deprotected at the nitrogen with
15 trifluoroacetic acid to give free amine 14. The latter
would be condensed separately with either
pentafluorophenylesters 6 or 8 to give 16 and 15
respectively, under conditions similar to those
described for 3. 15 and 16 would be deprotected in the
20 same manner as for 3 to yield desired analogs 4 and 5.

An inhibitor of the metabolism of purines and
pyrimidine like DON (6-diazo-5-oxo-norleucine) or its
aspartate-like 17, and glutamate-like 18 analogs would
25 be added to the series of substrates.

Analog 20 is transformed into compound 17 by treatment
with oxalyl chloride followed by diazomethane and
deprotection under known conditions to afford the
30 desired analogs. In addition, azotomycin is active only
after in vivo conversion to DON which will be released
after action of PSM on analogs 17, 18, and 19.

Representative compounds, 21 and 22, were designed
35 based on some of the specific effects and properties of
PSM, and the unique features of some newly discovered
cytotoxic molecules with now known mode of action. The

latter, referred to commonly as enediynes, like dynemycin A 23 and or its active analogs. The recent isolation of new natural products like Dynemycin A 23, has generated a tremendous and rapidly growing interest in the medical and chemical sciences. They have displayed cytotoxicities to many cancer cell lines at the sub-nanomolar level. One problem is they are very toxic, unstable, and non-selective. Although they have been demonstrated, in vitro, to exert their activity through DNA damage by a radical mechanism as described below, their high level of toxicity might imply that they should be able to equally damage anything in their path, from proteins to enzymes.

These molecules possess unusual structural features that provide them with exceptional reactivities. Dynemycin A 23 is relatively stable until the anthraquinone moiety is bio-reduced into hydroanthraquinone 24. This triggers a chain of events by which a diradical species 25 is generated as a result of a Bergman cycloaromatization^f. Diradical species 25 is the ultimate damaging edge of dynemycin A. It subtracts 2 (two) protons from any neighboring molecule or molecules (ie. DNA) producing radicals therein. These radicals in turn combine with molecular oxygen to give hydroperoxide intermediates that, in the case of DNA, lead to single and double strand incision, and consequent cell death. Another interesting feature was provided by the extensive work of many organic chemists who not only achieved the total synthesis of (+)-dynemycin A 23 and other enediynes. but also designed and efficiently prepared simpler yet as active analogs like 26.

Enediyne 26 is also triggerable and acts by virtue of the same mechanism as for 23. This aspect is very relevant to the present proposed study in that 27 (a

very close analog of 26) is connected to NAAG such that the NAAG-27 molecule, 21, would be inert anywhere in the body (blood, organs, normal prostate cells) except in the vicinity of prostate cancer, and metastatic cells. In this connection NAAG plays a multiple role:

- Solubilization and transport: analogs of 26-type are hydrophobic and insoluble in aqueous media, but with a water soluble dipeptide that is indigenous to the body, substrate 21 should follow the ways by which NAAG is transported and stored in the body.

- Recognition, guidance, and selectivity: Homologs of PSM are located in the small intestines and in the brain.

In the latter, a compound like 27 when attached to a multiply charged dipeptide like NAAG, has no chance of crossing the blood brain barrier. In the former case, PSM homolog concentration in the small intestines is in the brush border and not likely to be exposed to prodrugs in the serum. In addition, one could enhance the selectivity of delivery of the prodrug by local injection in the prostate.

26 and its analogs are established active molecules that portray the activity of dynemycin A. Their syntheses are described in the literature. The total synthesis of optically active 27 has been described⁶. The synthetic scheme that for the preparation of 28 is almost the same as that of 27. However, they differ only at the position of the methoxy group which is meta to the nitrogen in the case of 28. This requires an intermediate of type 29 prepared by modification of the Myers' method.

Since NAAG is optically pure, its combination with

racemic material sometimes complicates purification of intermediates. In addition, to be able to modify the components of this system one at a time, optically pure intermediates of the type 21 and 22 are prepared. 27
5 was prepared in 17 steps starting from commercially available material. Another interesting feature of 27 is demonstrated in a very close analog 26, it possesses two(2) triggers as shown by the arrows.

10 The oxygen and the nitrogen can both engender the Bergman cycloaromatization and hence the desired damage. The simple protection deprotection
15 manipulation of either functionality should permit the selective positioning of NAAG at the nitrogen or at the oxygen centers. PSM should recognize the NAAG portion of 21 or 22, then it would remove the glutamic acid moiety. This leaves 27 attached to N-acetylaspartate.

20 Intramolecular assisted hydrolysis of systems like N-acetylaspartyle is well documented in the literature. The aminoacid portion should facilitate the hydrolysis of such a linkage. In the event this would not work when NAAG is placed on the nitrogen, an alternative
25 would be to attach NAAG to the oxygen giving rise to phenolic ester 22 which is per se labile and removable under milder conditions. PSM specific substrates can be designed that could activate pro-drugs at the site of prostatic tumor cells to kill those cells.

optimized primers for detection of PSM mRNA in samples would preferably contain sequences hybridizing across the intro/exon junctions which are as follows:

```
5
      EXON 1      Intron 1
1F. strand
CGGCTTCCTCTTCGG
cggcttcctcttcgg taggggggcgcctcgcgag...tatttttca
10
1R. strand
...ataaaaagtCACCAA

      Exon 2      Intron 2
15 2F. strand
ACATCAAGAAGTTCT
acatcaagaagttct caagtaagtccatactcgaag...
20
2R. strand
...caagtggtcATATATTAAAATG

      Exon 3      Intron 3
3F. strand
GAAGATGGAAATGAG
25 gaagatggaaatgag gtaaaatataaataaataaataa...
3R.
...TAAAAGTTGTGTAGT

      Exon 4      Intron 4
30 4F. strand
AAGGAATGCCAGAGG
aaggaatgccagagg taaaaacacagtgaacaaa...
35
4R. strand
...agagttgCCGCTAGATCACA
```

EXAMPLE 13:

GENOMIC ORGANIZATION OF PSM EXON/INTRON JUNCTION
SEQUENCES

5
10
15
20
RNA is synthesized and then processed by having variable numbers of variable sized fragments cut out and remain in the nucleus (introns) and the remaining fragments (exons) joined together and transported out of the nucleus (mRNA) for use in translation into protein in the cytoplasm. This mRNA is what make the unique protein products of the cell, proteins of specialized cells are often made in a great abundance as are their respective coding mRNA's. These tissue specific mRNA's can be reverse transcribed (RT) into DNA by reverse transcriptase and amplified for detection by polymerase chain reaction (PCR) technology and thus the technique is called RT-PCR. If DNA is a contaminant of the MRNA fraction it would contain the message even though it was not being transcribed.

25
30
35
Knowledge of the intron exon junctions allows for the selection of primer pairs that cross an intron junction and thus allow the determination of DNA contamination of the RNA preparation, if present. If the intron junction were large it would be unlikely to be amplified with primers, while if the intron junction were small it would still produce a fragment that would be much larger than the predicted fragment size which is based on the cDNA sequence. Thus knowledge of the intron/exon junctions provides a control to determine if the RT-PCR product is contaminated with DNA. Another form of DNA that could also be amplified undesirably if present as a contaminant are pseudo genes, which are intronless forms of the mRNA that reside as DNA but are not expressed as RNA. Thus,

Exon 5 Intron 5

5F. strand
CAGAGGAAATAAGGT
cagaggaaataaggt aggtaaaaattatctctttttt...

5 5R. strand ...gtgttttctATTTTACGGGT

10 Exon 6 Intron 6

6F. strand
GTTACCCAGCAAATG
gttaccagcaatg gtgaatgatcaatccttgaat...

15 6R. strand ...aaaaaaagtTTATACGAATA

 Exon 7 Intron 7

20 7F. strand
ACAGAAGCTCCTAGA
acagaagctcctaga gtaagtttgtaagaaaccargg...

 7R. strand ...aaacacaggttatcTTTTACCCA

25 Exon 8 Intron 8

8F. strand
AAACTTTTCTACACA
aaacttttctacaca gttaagagactatataaatttta...

30 8R. strand aaacgtaatcaTTTTCAGTTCTAC

 Exon 9 Intron 9

35 9F. strand
AGCAGTGGAAACCAG
agcagtggaaccag gtaaaggaatcgtttgctagca...

 9R. strand ...aaagaTGTCTATACAGTAA

Exon 10 Intron 10
10F. Strand
CTGAAAAAGGAAGG
ctgaaaaaggaagg taatacaaacaaatagcaagaa...
5

Exon 11 Intron 11
11F. Strand
TGAGTGGGCAGAGG
10 agagg ttagttggtaatttgctataatata...

Exon 12 Intron 12
12F. strand
15 ATCTATAGAAGG
gtagtttct gaaaaataagaaaagaatagat...

Exon 13 Intron 13
20 13F. strand
CTAACAAAAGAG
agggcttttcagct acacaaattaaaagaaaaaag...

Exon 14 Intron 14
25 14F. strand
GTGGCATGCCCAGG
gtggcatgcccagg taaataaatgaatgaagtttcca...

Exon 15 Intron 15
30 15F. strand
CTAAAAATTGGC
aatttgtttgtttcc tacagaaaaaacaacaaaaca...
35

Exon 16 Intron 16

16F. strand
CAGTGTATCATTTG
cagtgtatcatttg gtatgttacccttcctttttcaaatt...

5

16R. strand ...aaagtcTAAGTGAAAA

Exon 17 Intron 17

10 17F. strand
TTTGACAAAAGCAA
tttgacaaaagcaa gtatgttctacatatatgtgcatat...

15

17R. strand ...aaagagtcGGGTTATCAT

Exon 18 Intron 18

18F. strand
GGCCTTTTTATAGG
20 ggcctttttatagg taaganaagaaaatatgactcct...

18R. strand ...aatagttgGTACAGTAGATA

25 Exon 19 Intron 19

19F. strand
GAATATTATATATA
gaatattatatata gttatgtgagtgtttatatatgtgtgt...

30

Notes: F: Forward strand
 R: Reverse strand

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- 35

What is claimed is:

1. An isolated nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen.
2. An isolated DNA of claim 1.
3. An isolated cDNA of claim 2.
4. An isolated RNA of claim 1.
5. An isolated DNA of claim 2 operatively linked to a promoter of RNA transcription.
6. A vector which comprises the nucleic acid of claim 1.
7. A host vector system for the production of a polypeptide having the biological activity of the alternatively spliced prostate-specific membrane antigen which comprises the vector of claim 6 and a suitable host.
8. A host vector system of claim 7, wherein the suitable host is a bacterial cell, insect cell, or mammalian cell.
9. An isolated nucleic acid comprising a promoter sequence normally associated with the transcription of a gene encoding a human prostate-specific membrane antigen.
10. An isolated polypeptide having the biological activity of an alternatively spliced prostate-specific membrane antigen.

11. An antibody which specifically binds to the polypeptide of claim 10.
- 5 12. The antibody of claim 11, wherein the antibody is monoclonal antibody.
13. The antibody of claim 11, wherein the antibody is polyclonal antibody.
- 10 14. The antibody of claim 11, wherein the antibody is labelled with a detectable marker.
- 15 15. The labelled antibody of claim 14, wherein the marker is radioactive, or colorimetric, luminescent, or fluorescent marker.
- 20 16. A method of detecting in a sample the presence of a nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen which comprises: a) obtaining a suitable sample; b) extracting RNA from the sample; c) contacting the RNA with reverse transcriptase under suitable conditions to obtain a cDNA; d) contacting the cDNA under hybridizing conditions with two
25 oligonucleotide primers,
 - 30 i) the first primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 3' of nucleotide 114 of such DNA sequence, with the proviso that the 3' end of the primer does not hybridize to any sequence located 5' of nucleotide 114, and
35 ii) the second primer being capable of specifically hybridizing to a sequence

5 within a DNA sequence encoding prostate
 specific membrane antigen located
 immediately 5' of nucleotide 381 of such DNA
 sequence, with the proviso that the 5' end
 of the primer does not hybridize to any
 sequence located 3' of nucleotide 381;

10 d) amplifying any cDNA to which the primers
 hybridize to so as to obtain amplification
 product; e) determining the size of the
 amplification product; f) comparing the size of
 the amplification product to the size of the
15 amplification product known to be obtained using
 the same primers with a non alternatively spliced
 human prostate specific membrane antigen, wherein
 a smaller amplification product is indicative of
 the presence of the alternatively spliced
 prostate specific membrane antigen, so as to
20 thereby detect the presence of the alternatively
 spliced human prostate-specific membrane antigen
 in the sample.

17. A method of detecting a prostate tumor cell in a
25 subject which comprises: which comprises: a)
 obtaining a suitable sample; b) extracting RNA
 from the sample; c) contacting the RNA with
 reverse transcriptase under suitable conditions
 to obtain a cDNA; d) contacting the cDNA under
 hybridizing conditions with two oligonucleotide
30 primers,

 i) the first primer being capable of
 specifically hybridizing to a sequence
 within a DNA sequence encoding prostate
35 specific membrane antigen located
 immediately 3' of nucleotide 114 of such
 DNA sequence, with the proviso that the 3'

end of the primer does not hybridize to any sequence located 5' of nucleotide 114, and

5 ii) the second primer being capable of specifically hybridizing to a sequence within a DNA sequence encoding prostate specific membrane antigen located immediately 5' of nucleotide 381 of such DNA sequence, with the proviso that the 5' end
10 of the primer does not hybridize to any sequence located 3' of nucleotide 381;

d) amplifying any cDNA to which the primers hybridize to so as to obtain amplification
15 product; e) determining the amount of the amplification product; f) comparing the amount of the amplification product to the amount of the amplification product known to be obtained using the same primers with a non alternatively
20 spliced human prostate specific membrane antigen, wherein a greater amount of the prostate specific membrane antigen is indicative of a prostate tumor cell in the subject, so as to thereby detect prostate tumor cell in the subject

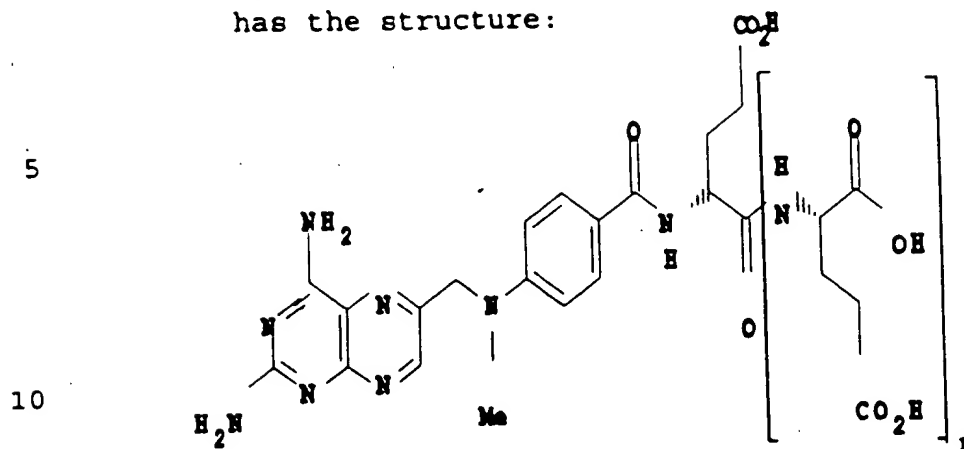
25

18. A compound comprising a conjugate of a cytotoxic agent and one or more amino acid residues, wherein each amino acid residue is glutamate or aspartate.

30

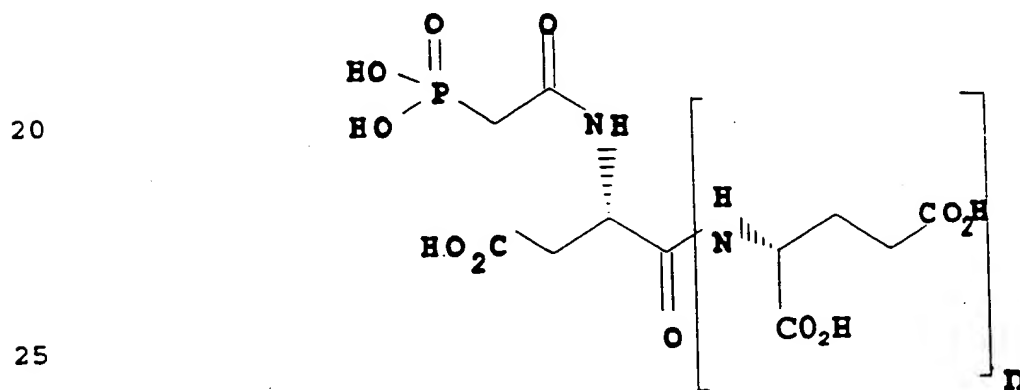
35

19. The compound of claim 18, wherein the compound has the structure:



wherein n is an integer from 1-10 inclusive.

- 15 20. The compound of claim 18, wherein the compound has the structure:

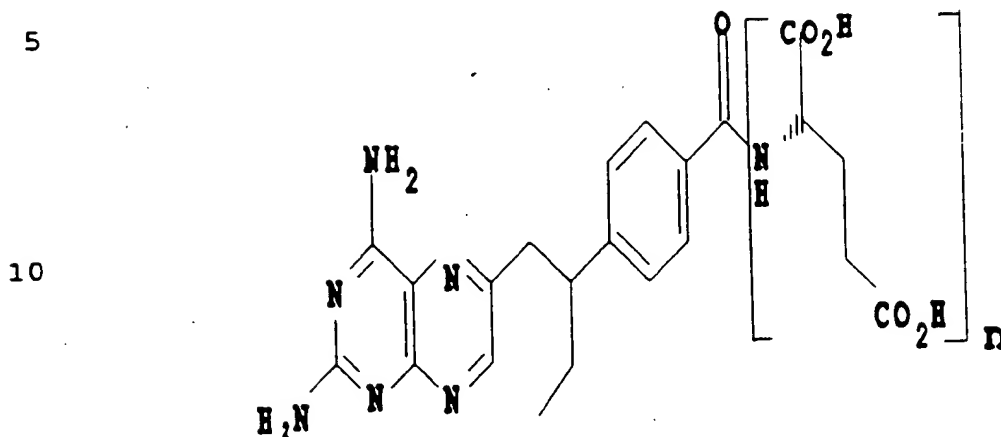


wherein n is an integer from 1-10 inclusive.

30

35

21. The compound of claim 18, wherein the compound has the structure:



wherein n is an integer from 1-10 inclusive.

22. A pharmaceutical composition comprising the compound of any of claims 18-21 in a therapeutically effective amount and a pharmaceutically acceptable carrier.
23. A method of making prostate cells suseptible to a cytotoxic chemotherapeutic agent, which comprises contacting the prostate cells with an the compound of any claims 18-21 in an amount effective to render the prostate cells suseptible to the cytotoxic chemotherapeutic agent.

PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

ABSTRACT OF THE INVENTION

5

This invention provides an isolated nucleic acid molecule encoding an alternatively spliced human prostate-specific membrane antigen. This invention provides an isolated nucleic acid comprising a promoter sequence normally associated with the transcription of a gene encoding a human prostate-specific membrane antigen. This invention provides an isolated polypeptide having the biological activity of an alternatively spliced prostate-specific membrane antigen.

10
15

This invention provides a method of detecting a nucleic acid encoding an alternatively spliced human prostate-specific membrane antigen and a method of detecting a prostate tumor cell in a subject.

20

Lastly, this invention provides a pharmaceutical composition comprising a compound in a therapeutically effective amount and a pharmaceutically acceptable carrier and a method of making prostate cells susceptible to a cytotoxic chemotherapeutic agent.

25

FIGURE 1A



FIGURE 1B

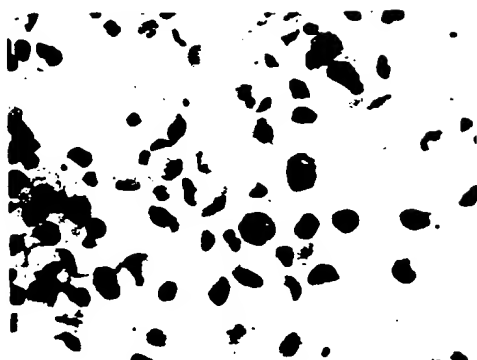


FIGURE 1C



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FIGURE 2

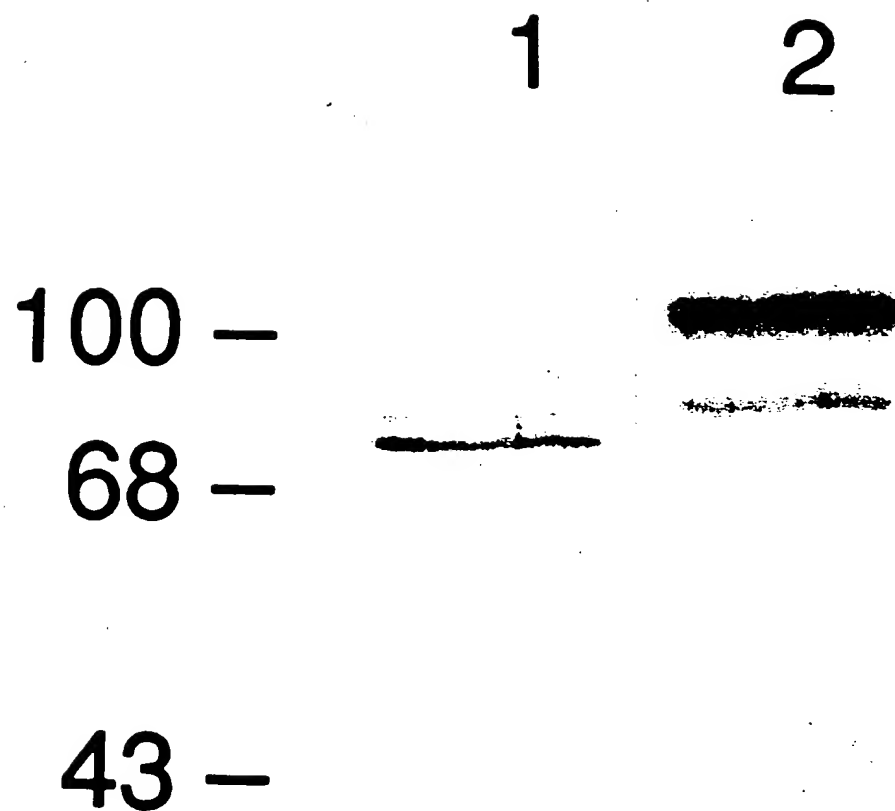


FIGURE 3

1 2 3 4

200 kDa —

100 kDa —

69 kDa —

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— PSM



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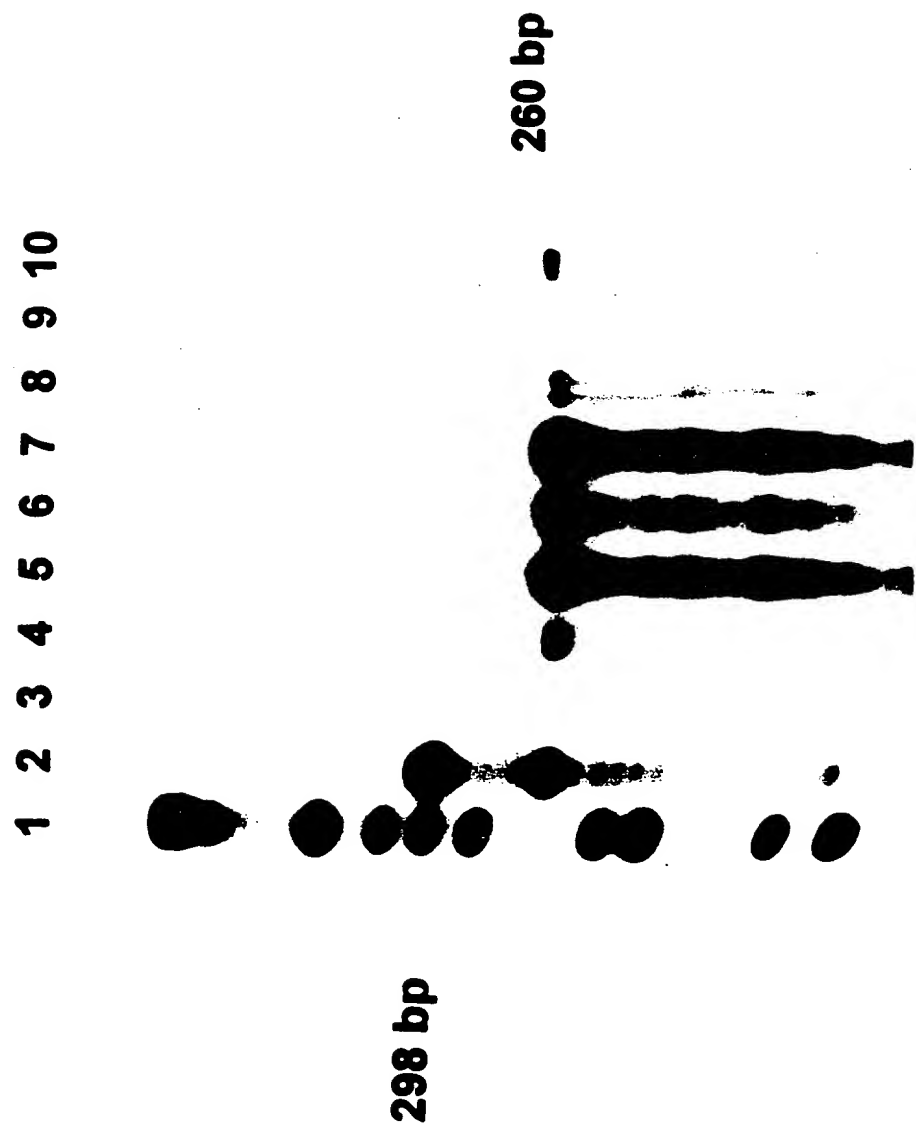
350

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

400

FIGURE 4

FIGURE 5



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FIGURE 6

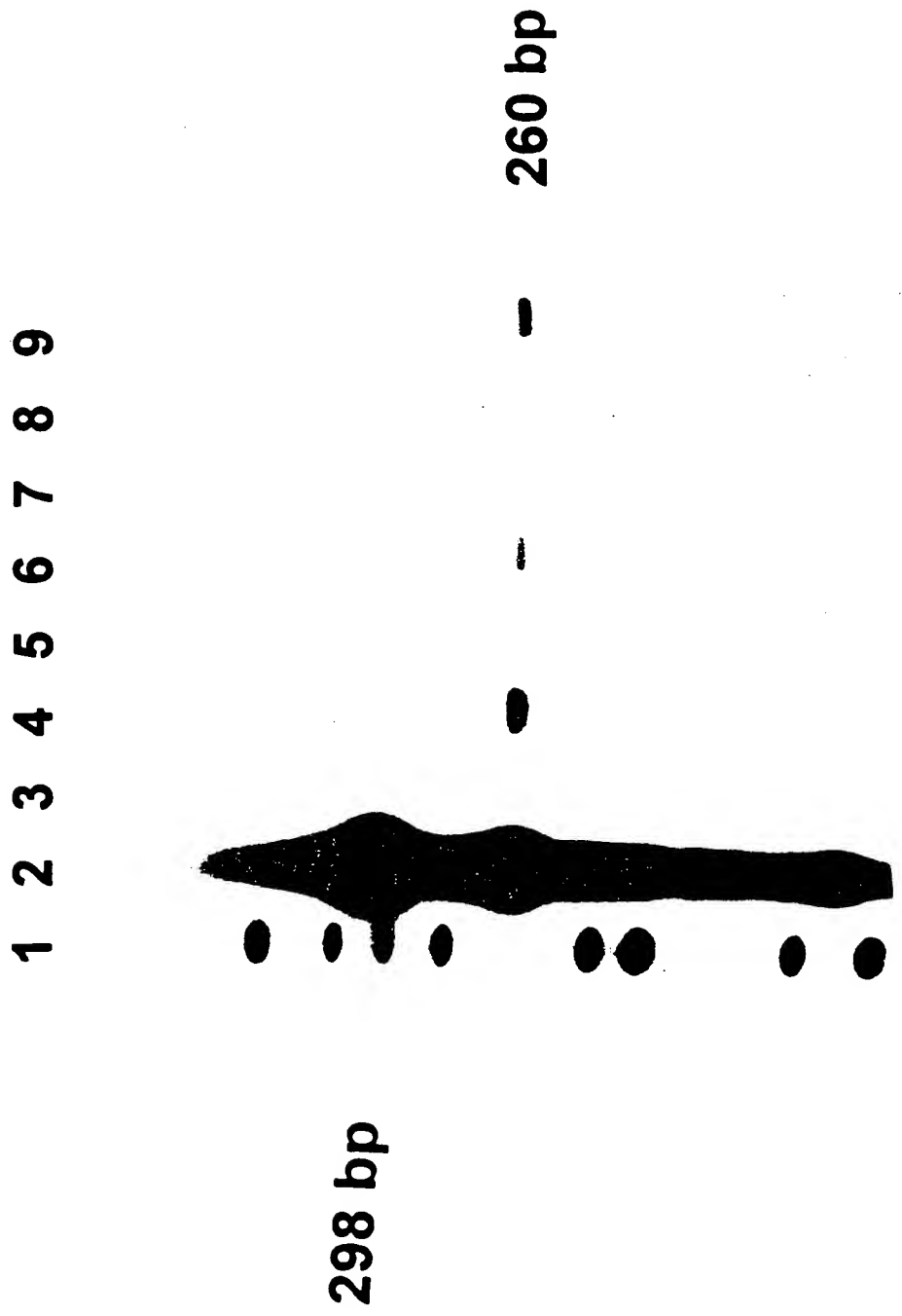


FIGURE 7

CELL LINE/TYPE	11p11.2-13 REGION	METASTATIC	PSM RNA DETECTED	PSM DNA DETECTED
LNCap			++	ND
HUMAN PROSTATE			++	ND
A9 (FIBROSARCOMA)	NO	NO	-	-
A9(11) (A9+HUM. 11)	YES	NO	-	REPEAT
AT6.1 (RAT PROSTATE)	NO	YES	-	-
AT6.1-11-c11	YES	NO	+	++
AT6.1-11-c12	NO	YES	-	-
R1564 (RAT MAMMARY)	NO	YES	-	-
R1564-11-c14	YES	YES	-	+
R1564-11-c15	YES	YES	-	REPEAT
R1564-11-c16	YES	YES	-	ND
R1564-11-c12	YES	YES	ND	+

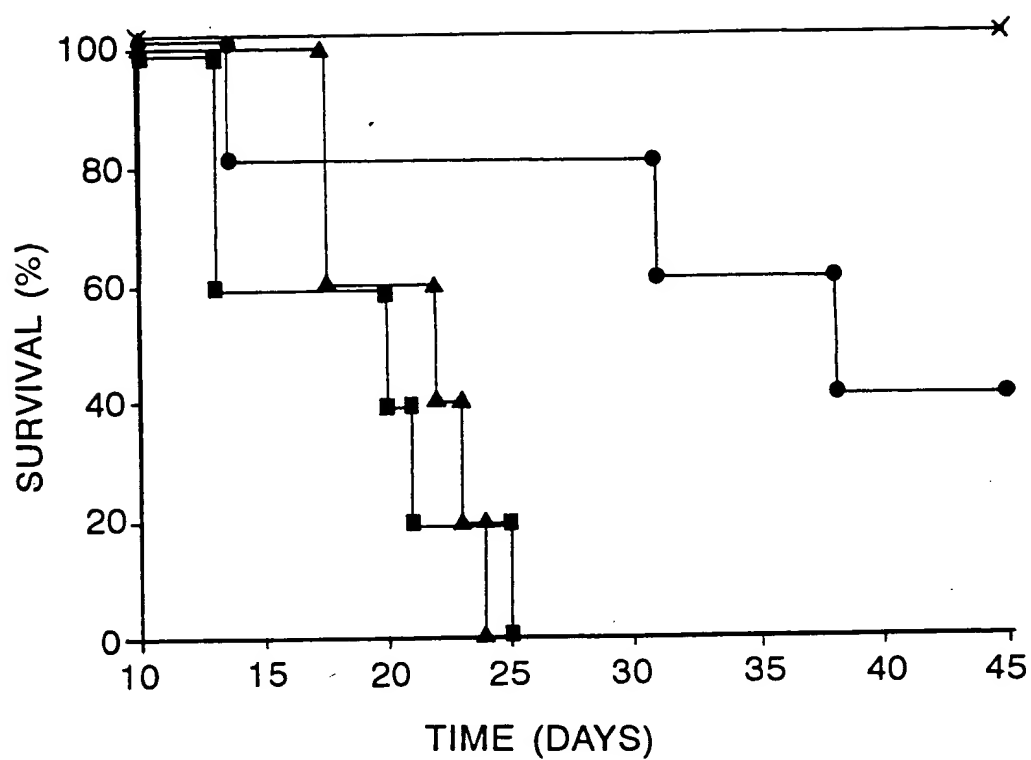
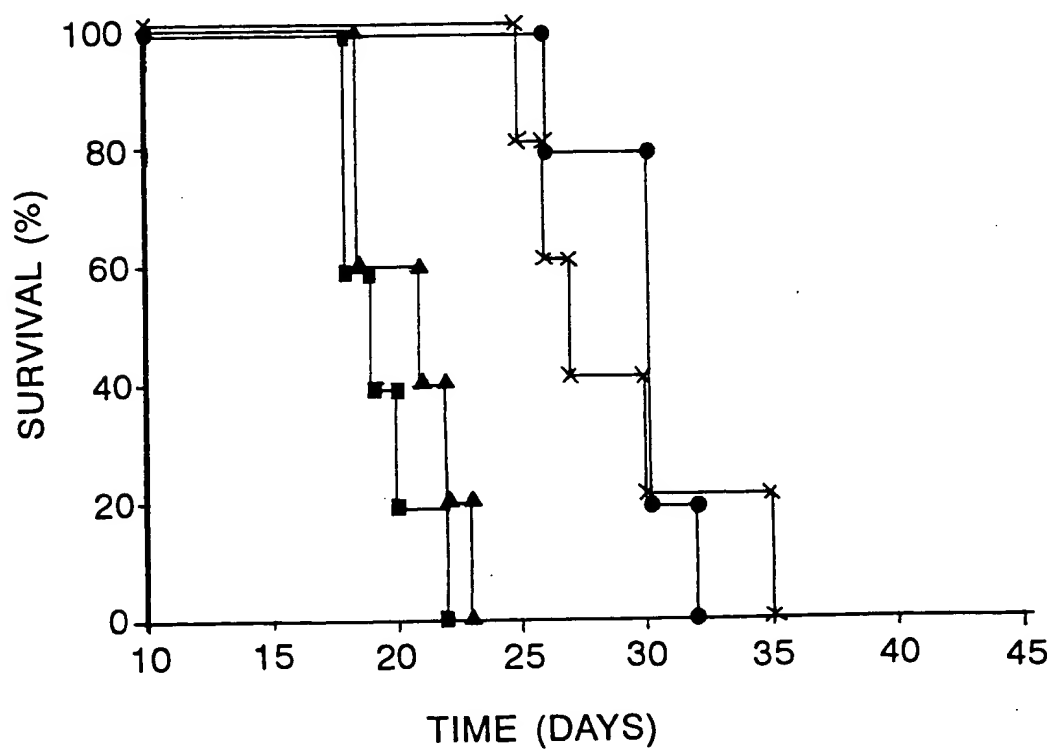
FIGURE 8A**FIGURE 8B**

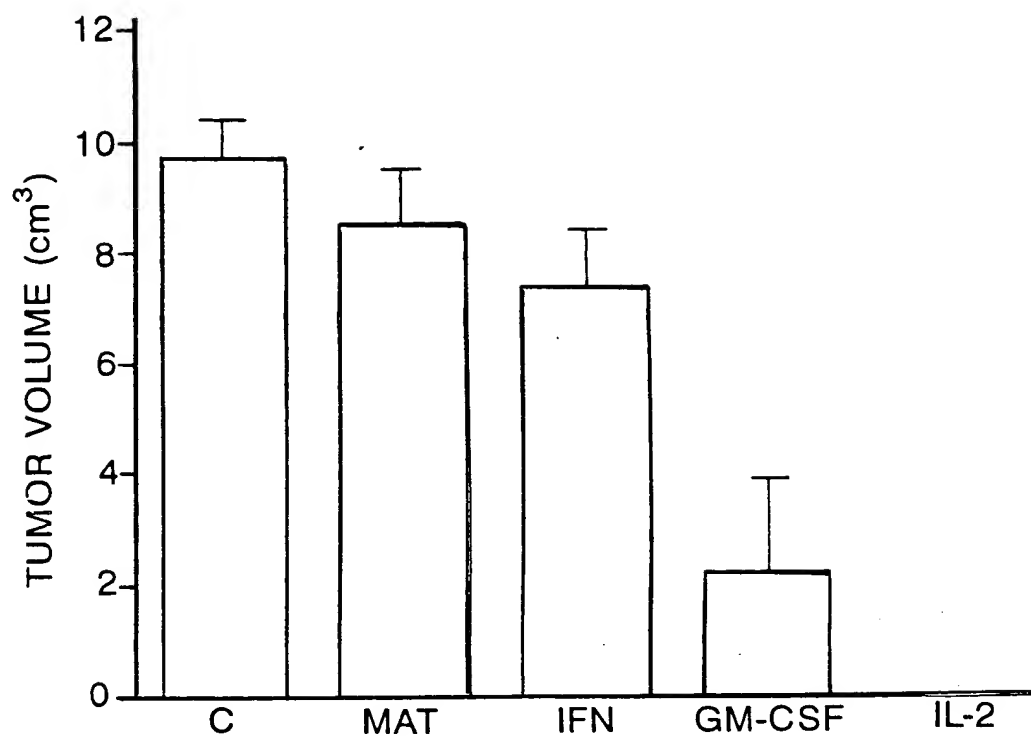
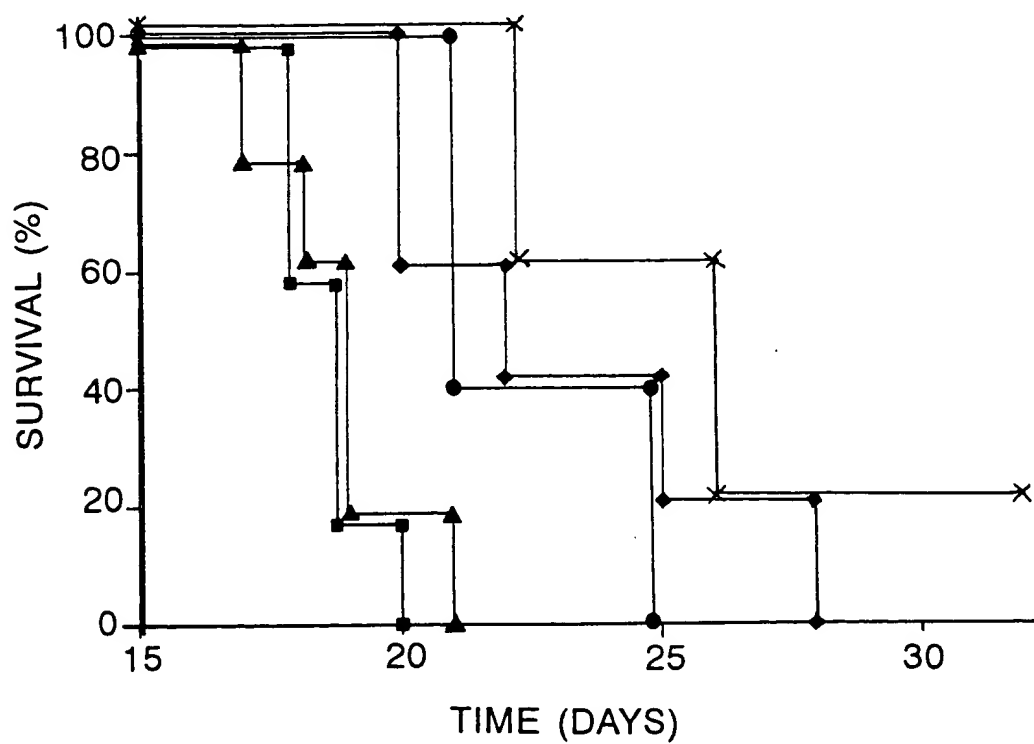
FIGURE 9A**FIGURE 9B**

FIGURE 10

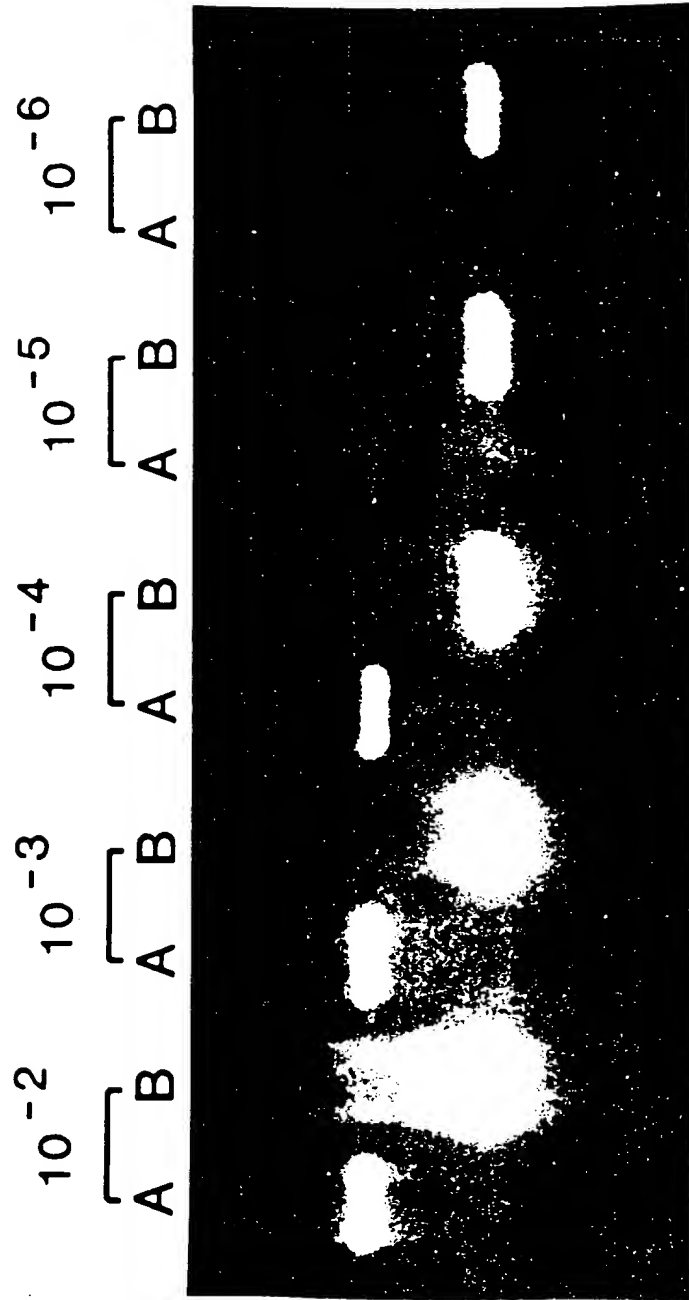


FIGURE 11

	10^{-3}	10^{-4}	10^{-5}	10^{-5}
	$\overbrace{A \quad B}$	$\overbrace{A \quad B}$	$\overbrace{A \quad B}$	$\overbrace{A \quad B}$
M	A	A	A	A
	B	B	B	B

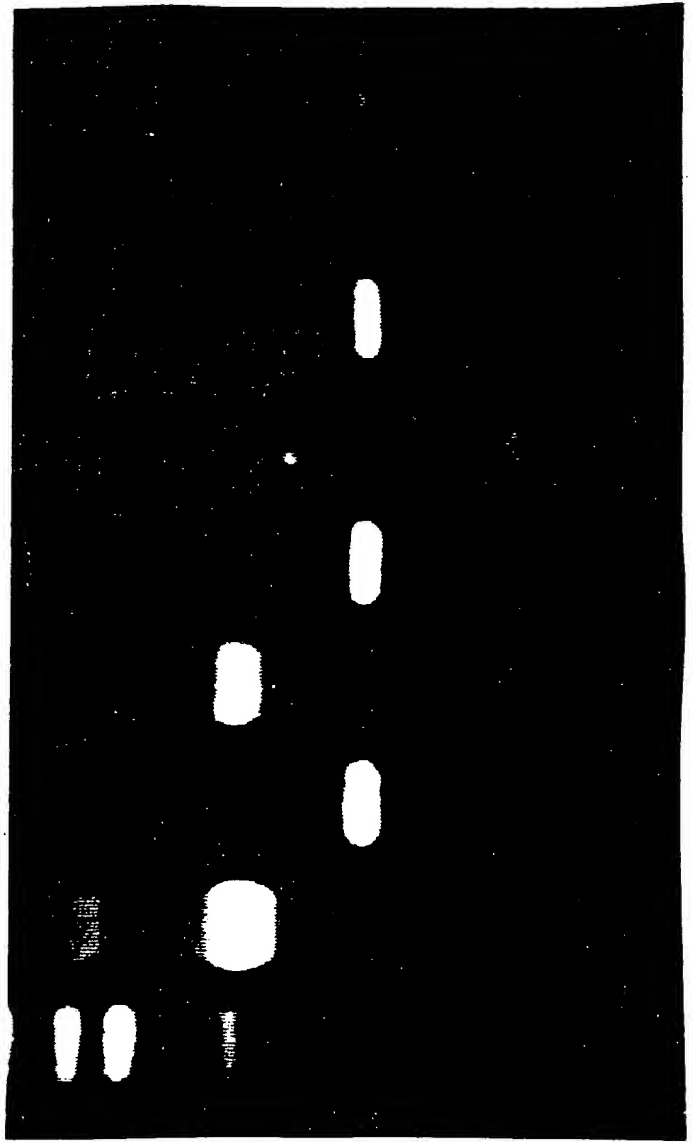


FIGURE 12

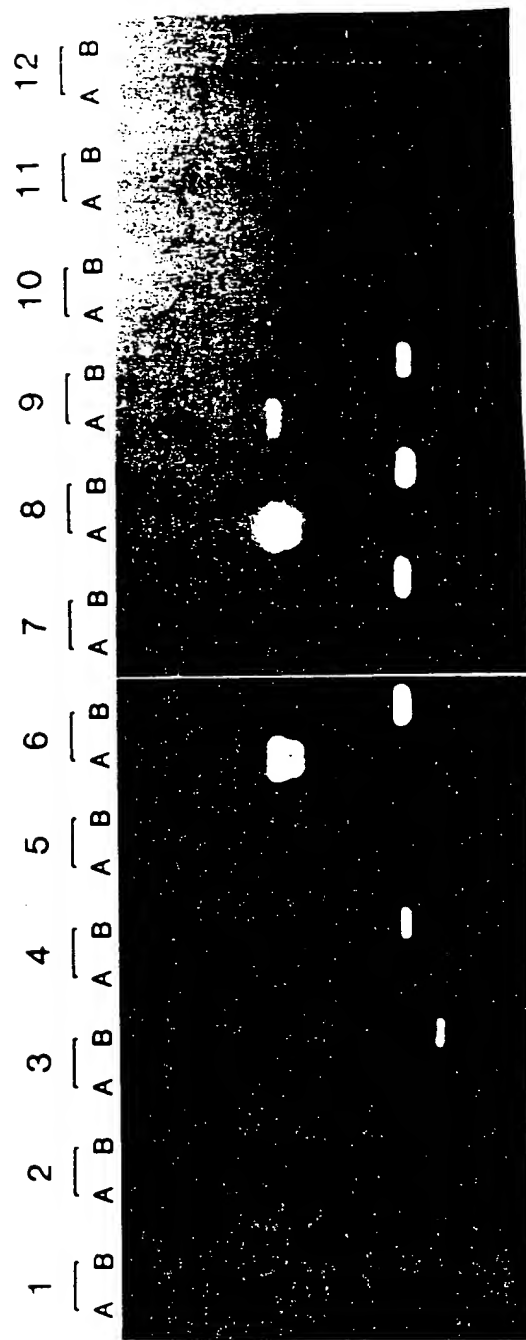


FIGURE 13



FIGURE 14

Patient	Stage	Treatment	PSA	PAP	PSA-PCR	PSM-PCR
1	T2NxMo	None	8.9	0.7	-	+
2	T2NoMo	RRP 7/93	6.1	-	-	+
3	T2CNoMo	PLND 5/93	4.5	0.1	-	+
4	T2BNoMo	RRP 3/92	NMA	0.4	-	+
5	T3NxMo	Proscar + Flutamide	51.3	1.0	-	+
6	Recur T3	I-125 1986	54.7	1.4	-	+
7	T3ANoMo	RRP 10/92	NMA	0.3	-	+
8	T3NxMo	XRT 1987	7.5	0.1	-	-
9	T3NxMo	Proscar + Flutamide	35.4	0.7	-	-
10	D2	S/P XRT Flutamide + Emcyt	311	4.5	+	+
11	D2	RRP 4/91 Lupron 10/92 Velban + Emcyt 12/92	1534	1.4	+	+
12	T2NoMo	RRP 8/91	NMA	0.5	-	+
13	T3NoMo	RRP 1/88 Lupron + Flutamide 5/92	0.1	0.3	-	-
14	D1	PLND 1989 XRT 1989	1.6	0.4	-	-
15	D1	Proscar + Flutamide	20.8	0.5	-	-
16	T2CNoMo	RRP 4/92	0.1	0.3	-	-

FIGURE 15A

	10	20	30	40	50	60
1	GCGCCTTAAA	AAAAAAAAAAC	TTTCTTGGA	AATGTCCAGC	TCTTGCTTAA	ATATAAAAAT
	CGCGGAATTT	TTTTTTTTTG	AAAGAACCTT	TTACAGGTCG	AGAACGAATT	TATATTTTTA
61	GAAAGGAAGA	AAGAGACTCT	CCTCTCTCCA	CTCCTATAAT	TATGAGGAAC	TTTTATTCAA
	CTTTCCTTCT	TTCTCTGAGA	GGAGAGAGGT	GAGGATATTA	ATACTCCTTG	AAAATAAGTT
121	CTCTGAAATT	CTATACAATC	TCTACAATAC	TCTACTGAAT	AAAAGCAGAG	CAGAAAAAGC
	GAGACTTTAA	GATATGTTAG	AGATGTTATG	AGATGACTTA	TTTTCGTCTC	GTCTTTTTCG
181	TSCGCTTTTT	TTCCATAGTC	GGGAATGCTT	GTCATCAGTG	TAAATCACCA	CCGCGCCCTT
	ACGCGAAAAA	AAGGTATCAG	CCCTTACGAA	CAGTAGTCAC	ATTTAGTGGT	GGCGCGGGAA
241	TTTCCTAAAG	AATATTATTG	TTATTAATAA	ACATGTAGGG	TATTATCCTC	CACTTACATT
	AAAGGATTTC	TTATAATAAC	AATAATTATT	TGTACATCCC	ATAATAGGAG	GTGAATGTAA
301	ACAAAACCAT	TTTTTAAAGC	CGGGCGTGGT	GGCTCACGCC	TGTAATCCCA	GCACTTTGGG
	TGTTTTGGTA	AAAAATTTTC	GCCCCACCA	CCGAGTGCGG	ACATTAGGGT	CGTGAAACCC
361	AGGCCCAGAC	AGGCGGATCA	CGAAGTCGAG	AAATCGAGAC	CATCCTGGCC	AACATGGTGA
	TCCGGGTCTG	TCCGCCTAGT	GCTTCAGCTC	TTTAGCTCTG	GTAGGACCGG	TTGTACCACT
421	AACCCCATCT	CTACTAAAAA	TACAAAAATT	AGCTGGGCGT	GGTGGCGGGC	TCCTGTAGTC
	TTGGGGTAGA	GATGATTTTT	ATGTTTTTTAA	TCGACCCGCA	CCACCGCCCG	AGGACATCAG
481	CCAGCTACTC	AGGAGGCTGA	GGCAGGAGAA	TCGCTTGAAC	CGGGGAGGCG	GAGGTTGCAG
	GGTCGATGAG	TCCTCCGACT	CCGTCTCTTT	AGCGAACTTG	GCCCCTCCGC	CTCCAACGTC
541	TCAGCCAAGA	TAGCGCCACT	GCACTGGAGC	CTGGTGACAG	AGTGAGACTC	CCTCAAGAAA
	AGTCGTTTCT	ATCGCGGTGA	CGTGACCTCG	GACCACTGTC	TCACTCTGAG	GGAGTTCTTT
601	GAAAGGAAGG	GAAGGGAAAG	GGAAGGAAGG	GGAGGGGAAG	GGAGGGGAGG	GGAGGGGAGG
	CTTCCTTTCC	CTTCCTTTTC	CCTTCCTTCC	CCTCCCCTTC	CCTCCCCTCC	CCTCCCCTCC
661	AAAGAAAAGA	ATACTGGAAC	TTGTTGAAGG	CAGAGACTTT	ATTTTCATAT	CCCGGCTATG
	TTTCTTTTCT	TATGACCTTG	AACAACCTCC	GTCTCTGAAA	TAAAAGTATA	GGGCCGATAC
721	TCTGGCTACT	GTCTTACGTA	ATAGATATAA	AATCAATCTT	GGTTGGATTA	ACCAGAAGAA
	AGACCGATGA	CAGAATGCAT	TATCTATATT	TTAGTTAGAA	CCAACCTAAT	TGGTCTTCTT

FIGURE 15B

781 TGAGAAGATA TATTCTGGTA AGTTGAATAC TTAGCACCCA GGGGTAATCA GCTTGGACAG
 ACTCTTCTAT ATAAGACCAT TCAACTTATG AATCGTGGGT CCCCATTAGT CGAACCTGTC

841 GACCAGGTCC AAAGACTGTT AAGAGTCTTC TGACTCCAAA CTCAGTGCTC CCTCCAGTGC
 CTGGTCCAGG TTTCTGACAA TTCTCAGAAG ACTGAGGTTT GAGTCACGAG GGAGGTCACG

901 CACAAGCAAA CTCCATAAAG GTATCCTGTG CTGAATAGAG ACTGTAGAGT GGTACAAAGT
 GTGTTGTTTT GAGGTATTTT CATAGGACAC GACTTATCTC TGACATCTCA CCATGTTTCA

961 AAGACAGACA TTATATTAAG TCTTAGCTTT GTGACTTCGA ATGACTTACC TAATCTAGCT
 TTCTGTCTGT AATATAATTC AGAATCGAAA CACTGAAGCT TACTGAATGG ATTAGATCGA

1021 AAATTTTCAGT TTTACCATGT GTAAATCAGG AAGAGTAATA GAACAAACCT TGAAGGGTCC
 TTTAAAGTCA AAATGGTACA CATTTAGTCC TTCTCAITAT CTTGTTTGA ACTTCCCAGG

1081 CAATGGTGAT TAAATGAGGT GATGTACATA ACATGCATCA CTCATAATAA GTGCTCTTTA
 GTTACCACTA ATTTACTCCA CTACATGTAT TGTACGTAST GAGTATTATT CACGAGAAAT

1141 AATATTAGTC ACTATTATTA GCCATCTCTG ATTAGATTG ACAATAGGAA CATTAGGAAA
 TTATAATCAG TGATAATAAT CCGTAGAGAC TAATCTAAAC TGTTATCCTT GTAATCCTTT

1201 GATATAGTAC ATTCAGGATT TTGTTAGAAA GAGATGAAGA AATTCCTTC CTTCCTGCCC
 CTATATCATG TAAGTCCTAA AACAATCTTT CTCTACTTCT TTAAGGGAAG GAAGGACGGG

1261 TAGGTCATCT AGGAGTTGTC ATGGTTCATT GTTGACAAAT TAATTTTCCC AAATTTTTCa
 ATCCAGTAGA TCCTCAACAG TACCAAGTAA CAACTGTTTA ATTAAAAGGG TTTAAAAAGT

1321 CTTTGCTCAG AAAGTCTACA TCGAAGCACC CAAGACTGTA CAATCTAGTC CATCTTTTTC
 GAAACGAGTC TTTGAGATGT AGCTTCGTGS GTTCTGACAT GTTAGATCAG GTAGAAAAAG

1381 CACTTAACTC ATACTGTGCT CTCCTTTTCT CAAAGCAAAC TGTTTGCTAT TCCTTGAATA
 GTGAATTGAG TATGACACGA GAGGGAAGA GTTTCGTTT CAAACGATA AGGAACTTAT

1441 CACTCTGAGT TTTCTGCCTT TGCCTACTCA GCTGGCCCAT GGCCCTTAAT GTTCTTCTC
 GTGAGACTCA AAAGACGGAA ACGGATGAGT CGACCGGTA CCGGGGATTA CAAAGAAGAG

1501 ATCTCCACTG GGTCAAATCC TACCTGTACC TTATGGTTCT GTTAAAAGCA GTGCTTCCAT
 TAGAGGTGAC CCAGTTTAGG ATGGACATGG AATACCAAGA CAATTTTCGT CACGAAGGTA

1561 AAAGTACTCC TAGCAAATGC ACGGCCTCTC TCACGGATTA TAAGAACACA GTTTATTTTA
 TTTGATGAGG ATCGTTTACG TGCCGGAGAG AGTGCCTAAT ATTCTTGTGT CAAATAAAAT

1621 TAAAGCATGT AGCTATTCTC TCCCTCGAAA TACGATTATT ATTATTAAGA ATTTATAGCA
 ATTTCTGATA TCGATAAGAG AGGGAGCTTT ATGCTAATAA TAATAATTCT TAAATATCGT

1681 GGGATATAAT TTTGTATGAT GATTCTTCTG GTTAATCCAA CCAAGATTGA TTTTATATCT
 CCTATATTA AACATACTA CTAAGAAGAC CAATTAGGTT GGTTCCTAAT AAAATATAGA

1741 ATTACGTAAG ACAGTAGCCA GACATAGCCG GGATATGAAA ATAAAGTCTC TGCCCTCAAC
 TAATGCATTC TGTATCGGT CTGTATCGGC CCTATACTTT TATTTACAGAG ACGGAAGTGG

1801 AAGTTCCAGT ATTCTTTTCT TTCCTCCCTT CCCCTCCCTT CCTTCCCTT CCCCTTCCCT
 TTCAAGGTCA TAAGAAAAGA AAGGAGGGGA GGGGAGGGGA GGGGAAGGGGA GGGGAAGGAA

1861 CCCTTCCCTT TCCCTTCCCT TCTTTCTTGA GGGAGTCTCA CTCTGTCACC AGGCTCCAGT
 GGGAAAGGGA AGGGAAGGAA AGAAAGAACT CCCTCAGAGT GAGACAGTGG TCCGAGGTCA

FIGURE 15C

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1921 GCAGTGGCGC TATCTTGGCT GACTGCAACC TCGCCCTCCC CGGTTCAAGC GATTCTCCTG
 CGTCACCGCG ATAGAACCGA CTGACGTTGG AGGCGGAGGG GCCAAGTTCC CTAAGAGGAC

1981 CCTCAGCCTC CTGAGTAGCT GGGACTACAG GAGCCCGCCA CCACGCCACG CTAATTTTTG
 GGAGTCGGAG GACTCATCGA CCCTGATGTC CTCGGGCGGT GGTGCGGGTC GATTAATAAAC

2041 TATTTTATAGT AGAGATGGGG TTTCACCATG TTGGCCAGGA TGGTCTCGAT TTCTCGACTT
 ATAAAAATCA TCTCTACCCC AAAGTGGTAC AACCGGTCCT ACCAGAGCTA AAGAGCTGAA

2101 CGTGATCCGC CTGTCTGGGC CTCCCAAAGT GCTGGGATTA CAGGCGTGAG CCACCACGCC
 GCAC TAGGCG GACAGACCGG GAGGGTTTCA CGACCCTAAT GTCCGCACTC GGTGGTGGCG

2161 CGGCTTTAAA AAATGGTTTT GTAATGTAAG TGGAGGATAA TACCCTACAT GTTTATTAAT
 GCGGAAATTT TTTACCAAAA CATTACATTC ACCTCCTATT ATGGGATGTA CAAATAATTA

2221 AACATAATA TTCTTTAGGA AAAAGGGCGC GGTGGTGATT TACACTGATG ACAAGCATTC
 TTGTTATTAT AAGAAATCCT TTTCGCCGCG CCACCCTAA ATGTGACTAC TGTTCTGTAAG

2281 CCGACTATGG AAAAAAAGCG CAGCTTTTTTC TGCTCTGCTT TTATTCAGTA GAGTATTGTA
 GGCTGATACC TTTTTTTCGC GTCGAAAAAG ACGAGACGAA AATAAGTCAT CTCATAACAT

2341 GAGATTGTAT AGAATTTTCT AGTIGAATAA AAGTTCCTCA TAATTATAGG AGTGGAGAGA
 CTCTAACATA TCTTAAAGTC TCAACTTATT TTCAAGGAGT ATTAATATCC TCACCTCTCT

2401 GGAGAGTCTC TTTCTCTCCT TCATTTTTAT ATTTAAGCAA GAGCTGGACA TTTTCCAAGA
 CCTCTCAGAG AAAGAAGGAA AGTAAAAATA TAAATTCGTT CTCGACCTGT AAAAGGTTCT

2461 AAGTTTTTTT TTTTAAAGGC GCCTCTCAAA AGGGGCCGGA TTCTCTTCTC CTGGAGGCAG
 TTCAAAAAAA AAAAATTCGG CGGAGAGTTT TCCCCGGCCT AAAGGAAGAG GACCTCCGTC

2521 ATGTTGCCTC TCTCTCTCGC TCGGATTGGT TCAGTGCACT CTAGAAACAC TGCTGTGGTG
 TACAACGGAG AGAGAGAGCG AGCCTAACCA AGTCACGTGA GATCTTTGTG ACGACACCAC

2581 GAGAACTGG ACCCCAGGTC TGGAGCGAAT TCCAGCCTGC AGGGCTGATA AGCGAGGCAT
 CTCTTTGACC TGGGGTCCAG ACCTCGCTTA AGGTGCGACG TCCCGACTAT TCGCTCCGTA

2641 TAGTGAGATT GAGAGAGACT TTACCCCGCC GTGGTGTTTG GAGGGCGCGC AGTAGAGCAG
 ATCACTCTAA CTCTCTCTGA AATGGGGCGG CACCACCAAC CTCGCGCGCG TCATCTCGTC

2701 CAGCACAGGC CCGGGTCCCG GGAGGCCGGC TCTGCTCGCG CCGAGATGTG GAATCTCCTT
 GTCGTGTCCG CGCCCAAGGC CCTCCGGCCG AGACGAGCGC GGCTCTACAC CTTAGAGGAA

2761 CACGAAACCG ACTCGGCTGT GGCCACCGCG CGCCGCCCGC GCTGGCTGTG CGCTGGGGCG
 GTGCTTTGGC TGAGCCGACA CCGGTGGCGC GCGGCGGGCG CGACCGACAC GCGACCCCGC

2821 CTGGTGCTGG CCGGTGGCTT CTTTCTCCTC GGCTTCCTCT TCGGTAGGGG GGCGCCTCGC
 GACCACGACC GCCCACCGAA GAAAGAGGAG CCGAAGGAGA AGCCATCCCC CCGCGGAGCG

2881 GGAGCAAACC TCGGAGTCTT CCCCCTGGTG CCGCGGTGCT GGGACTCGCG GGTGAGCTGC
 CCTCGTTTGG AGCCTCAGAA GGGGCACCAC GCGGCCACGA CCTGAGCGC CCAGTGAGC

2941 CGAGTGGGAT CCTGTGCTG GTCTTCCCCA GGGGCGGCGA TTAGGGTCGG GGTAAATGTG
 GCTCACCCTA GGACAACGAC CAGAAGGGGT CCCCAGCGCT AATCCCAGCC CCATTACACC

3001 GGTGAGCACC CCTCGAG
 CCACTCGTGG GGAGCTC

FIGURE 15D

- 2401 GGAGAGTCTC TTTCTTCCTT TCATTTTTAT ATTTAAGCAA GAGCTGGACA TTTTCCAAGA
 CCTCTCAGAG AAAGAAGGAA AGTAAAAATA TAAATTCGTT CTCGACCTGT AAAAGGTTCT

- 2461 AAGTTTTTTT TTTTAAAGGC GCCTCTCAA AGGGGCCGGA TTTCCTTCTC CTGGAGGCAG
 TTCAAAAAAA AAAAATTCCG CGGAGAGTTT TCCCCGGCCT AAAGGAAGAG GACCTCCGTC

- 2521 ATGTTGCCTC TCTCTCTCGC TCGGATTGGT TCAGTGCACT CTAGAAACAC TGCTGTGGTG
 TACAACGGAG AGAGAGAGCG AGCCTAACCA AGTCACGTGA GATCTTTGTG ACGACACCAC

- 2581 GAGAAACTGG ACCCCAGGTC TGGAGCGAAT TCCAGCCTGC AGGGCTGATA AGCGAGGCAT
 CTCCTTGACC TGGGGTCCAG ACCTCGCTTA AGGTCGGACG TCCCGACTAT TCGCTCCGTA

- 2641 TAGTGAGATT GAGAGAGACT TTACCCCGCC GTGGTGGTTG GAGGGCGCGC AGTAGAGCAG
 ATCACTCTAA CTCTCTCTGA AATGGGGCGG CACCACCAAC CTCCCGCGCG TCATCTCGTC

- 2701 CAGCACAGGC GCGGGTCCCG GGAGGCCGCG TCTGCTCGCG CCGAGATGTG GAATCTCCTT
 GTCGTGTCCG CGCCAGGGC CCTCCGGCCG AGACGAGCGG GGCTCTACAC CTTAGAGGAA

- 2761 CACGAAACCG ACTCGGCTGT GGCCACCGCG CGCCGCCCGC GCTGGCTGTG CGCTGGGGCG
 GTGCTTTGGC TGAGCCGACA CCGGTGGCGC GCGGCGGGCG CGACCGACAC GCGACCCCGC

- 2821 CTGGTGCTGG CGGGTGGCTT CTTTCTCCTC GCCTTCCTCT TCGGTAGGGG GGCGCCTCGC
 GACCACGACC GCCACCGAA GAAAGAGGAG CCGAAGGAGA AGCCATCCCC CCGCGGAGCG

- 2881 GGAGCAAACC TCGGAGTCTT CCCCCTGGTG CCGCGGTGCT GGGACTCGCG GGTCAGCTGC
 CCTCGTTTGG AGCCTCAGAA GGGGCACCAC GCGGCCACGA CCCTGAGCGC CCAGTCGACG

- 2941 CGAGTGGGAT CCTGTTGCTG GTCTTCCCCA GGGGCGGCGA TTAGGGTCGG GGTAATGTGG
 GCTCACCTA GGACAACGAC CAGAAGGGGT CCCCGCCGCT AATCCCAGCC CCATTACACC

- 3001 GGTGAGCACC CCTCGAG
 CCACTCGTGG GGAGCTC

FIGURE 16

Potential binding sites on the PSM promoter*

Site	Seq	**Location	#nt matched
AP1	TKAGTCA	-1145	7/7
E2-RS	ACCNNNNNNGGT	-1940	12/12
		-1951	12/12
GHF	NNNTAAATNNN	-580	11/11
		-753	11/11
		-1340	11/11
		-1882	11/11
		-1930	11/11
		-1979	11/11
		-2001	11/11
		-2334	11/11
		-2374	11/11
		-2591	11/11
		-2620	11/11
		-2686	11/11
JVC repeat	GGGNGGRR	-1165	8/8
		-1175	8/8
		-1180	8/8
		-1185	8/8
		-1190	8/8
NFkB	GGGRHTYYHC	-961	10/10
uteroglobi	RYYWSGTG	-250	8/8
		-921	8/8
		-1104	8/8
IFN	AAWAANGAAAGGR590	13/13	Cell 41:509 (1985)

FIGURE 17

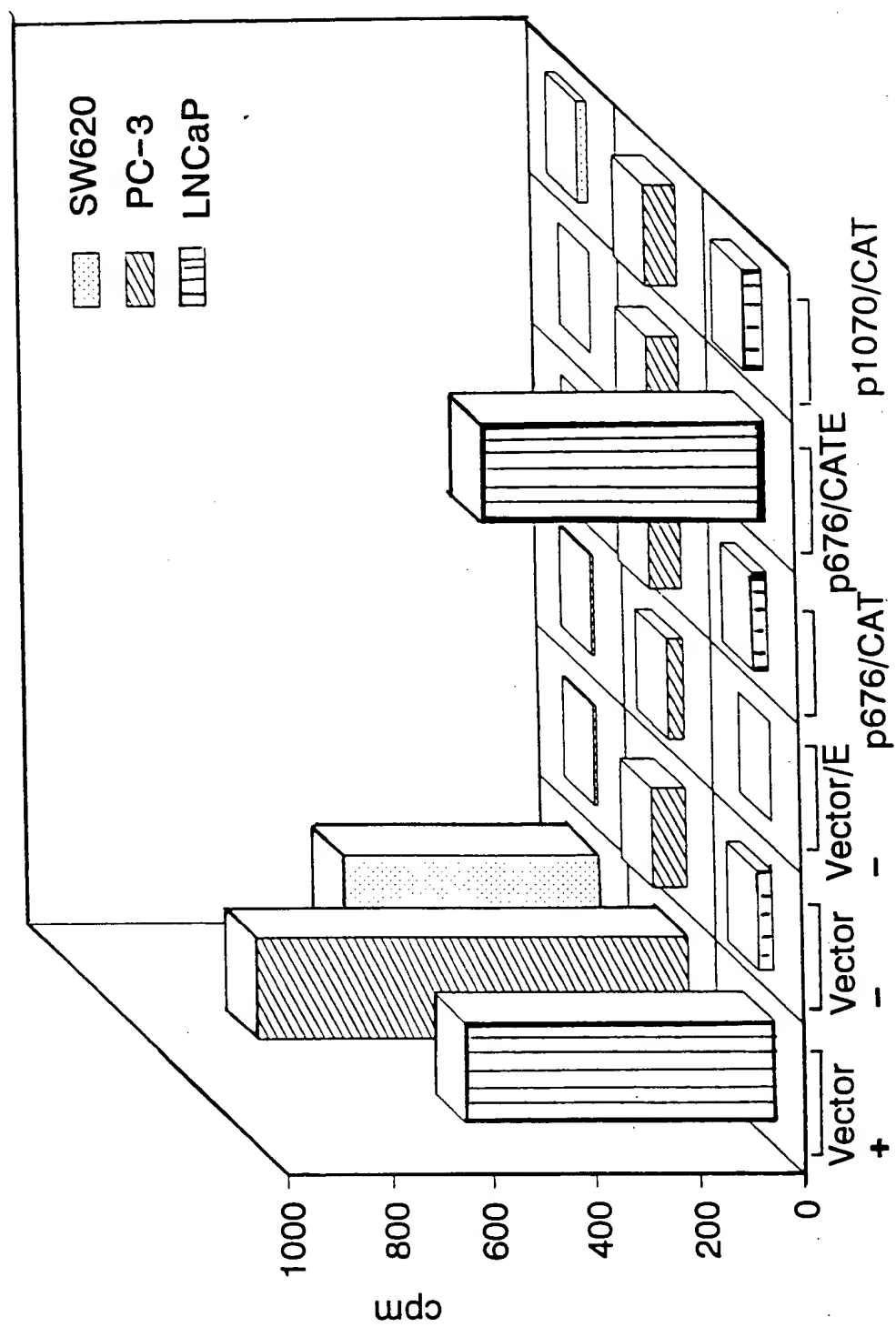


FIGURE 18

CTCAAAAGGGCCGGATTTCCT
TCT TGGAGGAGATGTTGCCCTCTCTCTCTCGGATTGGTTCAGTGCACCTCTAGAACACTGCTGTGTGGAGAAACT
GGACCCC AGG TCTGGAGCGAATTCCA GCCTGCAGGGCTGATAAGCGAGGCATTAGTGAGATTGAGAGAGACTTTACCC
CGCGGTGGTTGGAGGGCGGCAGT AGAGCAGCAGCACAGCGCGGTCCCGGAGGCCGCTCTGCTCGCGCGCGAG

ATG TGG AAT CTC CTT CAC GAA ACC GAC TCG GCT GTG GCC ACC GCG CCG CCG CCG CTG CTG

Met Trp Asn Leu Leu His Glu Thr Asp Ser Ala Val Ala Ala Arg Arg Pro Arg Trp Leu

TGC GCT GGG GCG CTG GTG CTG GCG GGT GGCTTC TTT CTC CTC GGC TTC CTC TTC GGA TGG TTT

Cys Ala Gly Ala Leu Val Leu Ala Gly Gly Phe Phe Leu Leu Gly Phe Leu Phe Gly Trp Phe

ATA AAA TCC TCC AAT GAA GCT ACT AAC ATT ACT CCA AAG CAT AAT ATG AAA GCA TTT TTG GAT GAA

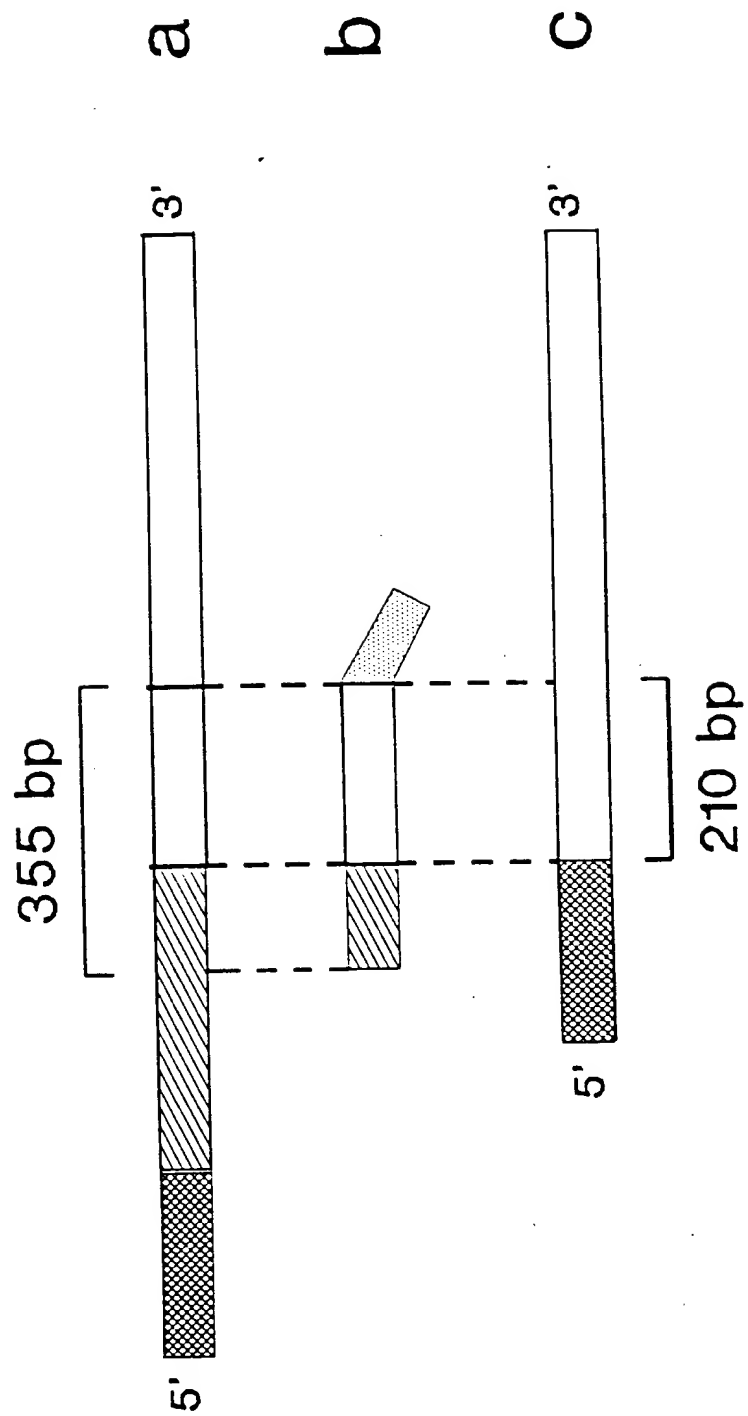
Ile Lys Ser Ser Asn Glu Ala Thr Asn Ile Thr Pro Lys His Asn Met Lys Ala Phe Leu Asp Glu

*

TGG AAA GCT GAG AAC ATC AAG AAG TTC TTA TAT AAT TTT ACA CAG ATA CCA CAT TTA GCA GGA ACA

Leu Lys Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Gln Ile Pro His Leu Ala Gly Thr

FIGURE 19



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FIGURE 20

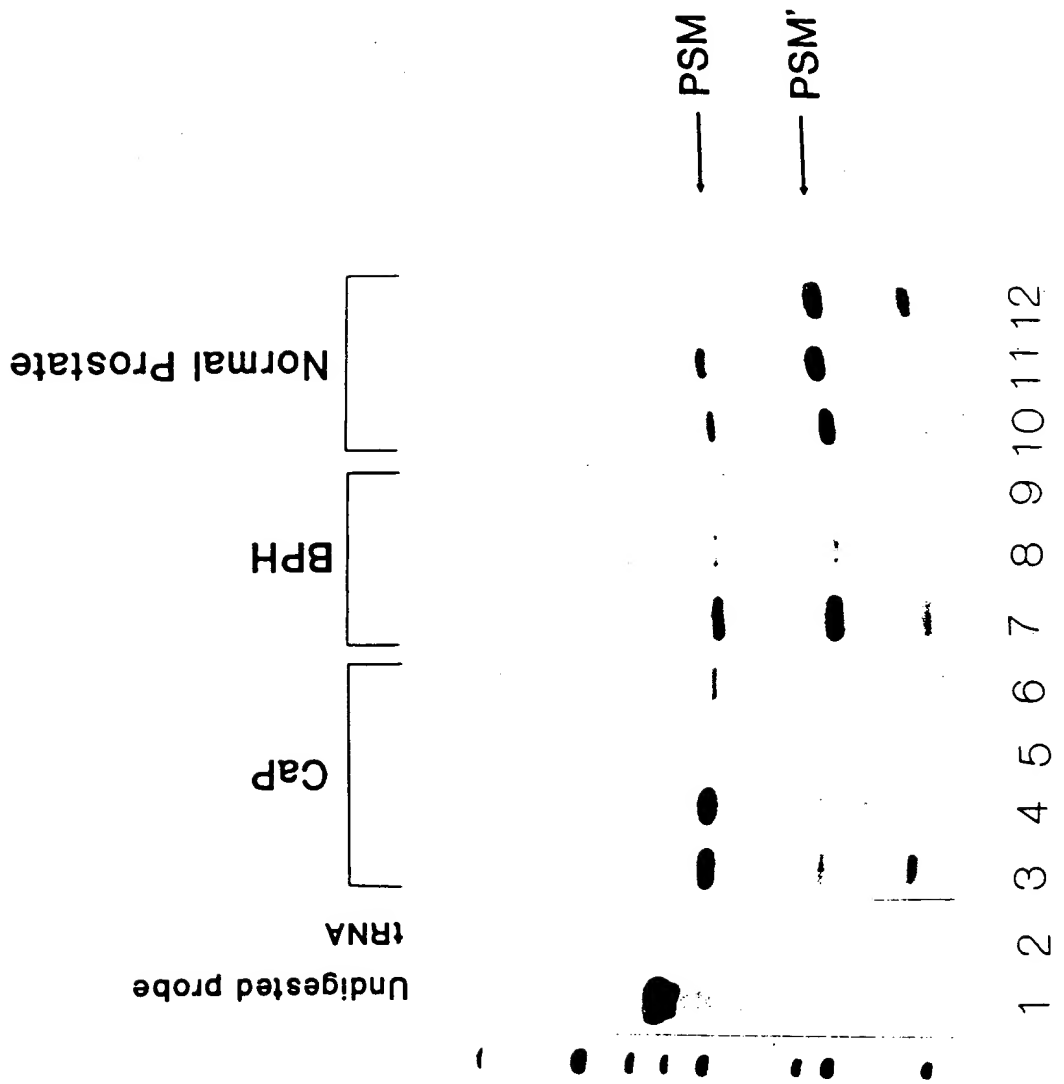
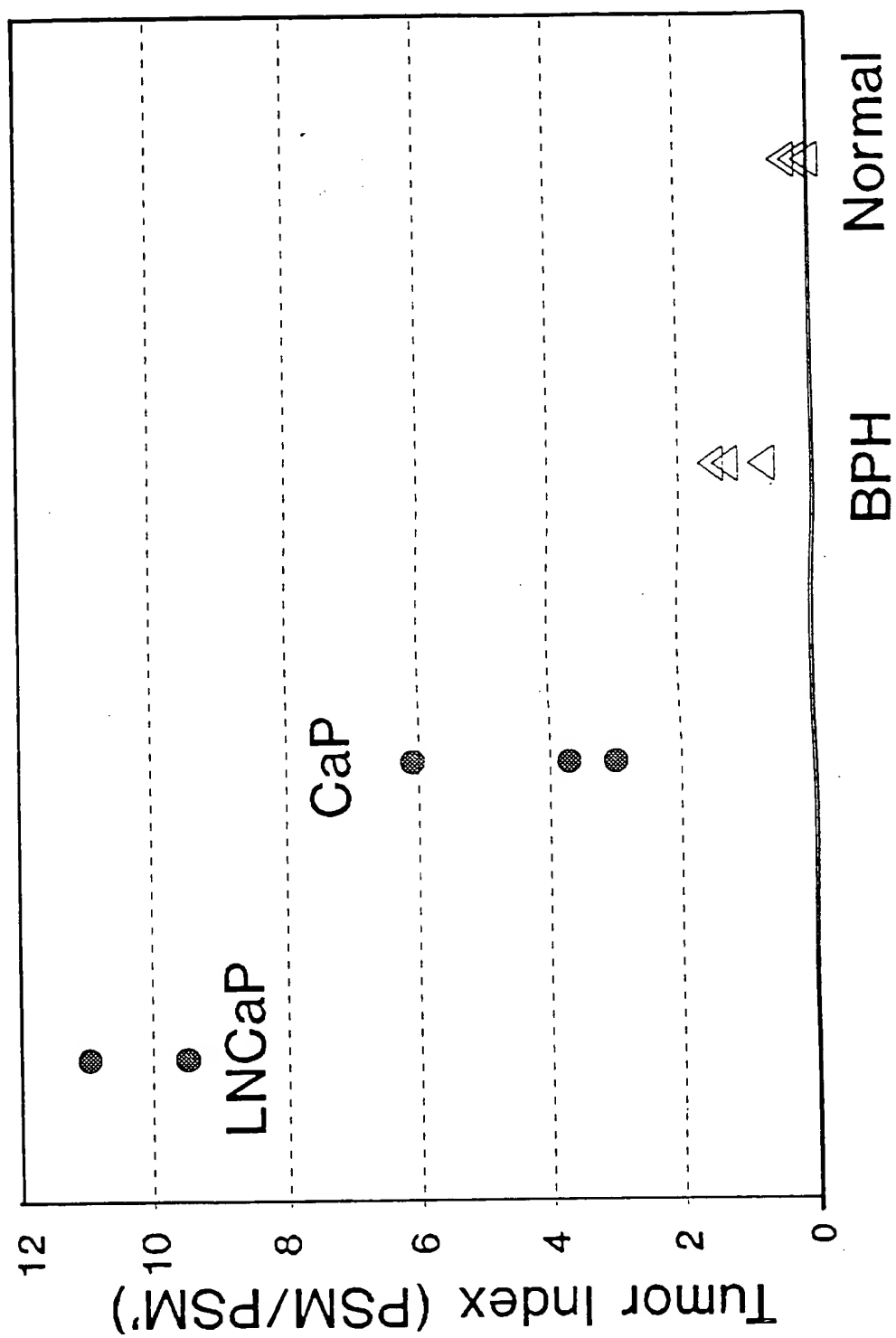


FIGURE 21



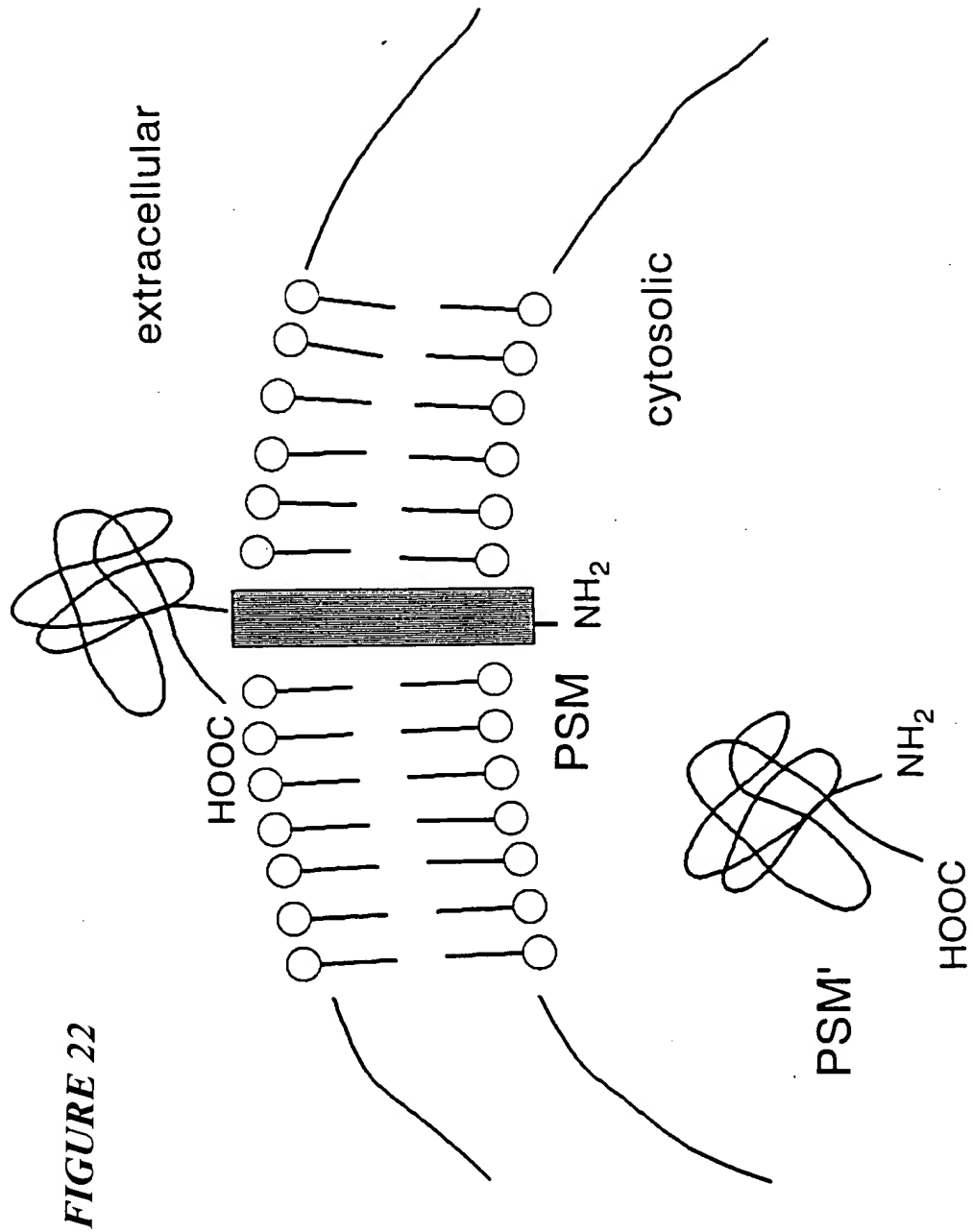


FIGURE 23

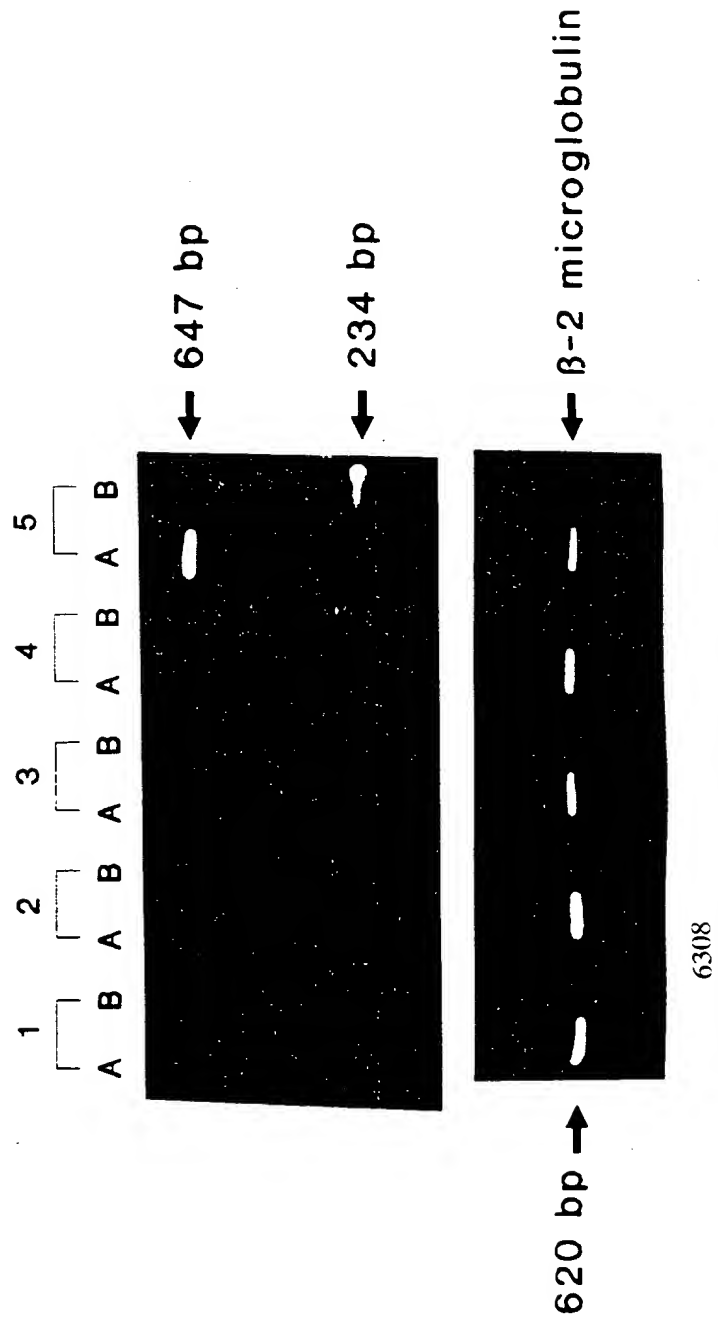
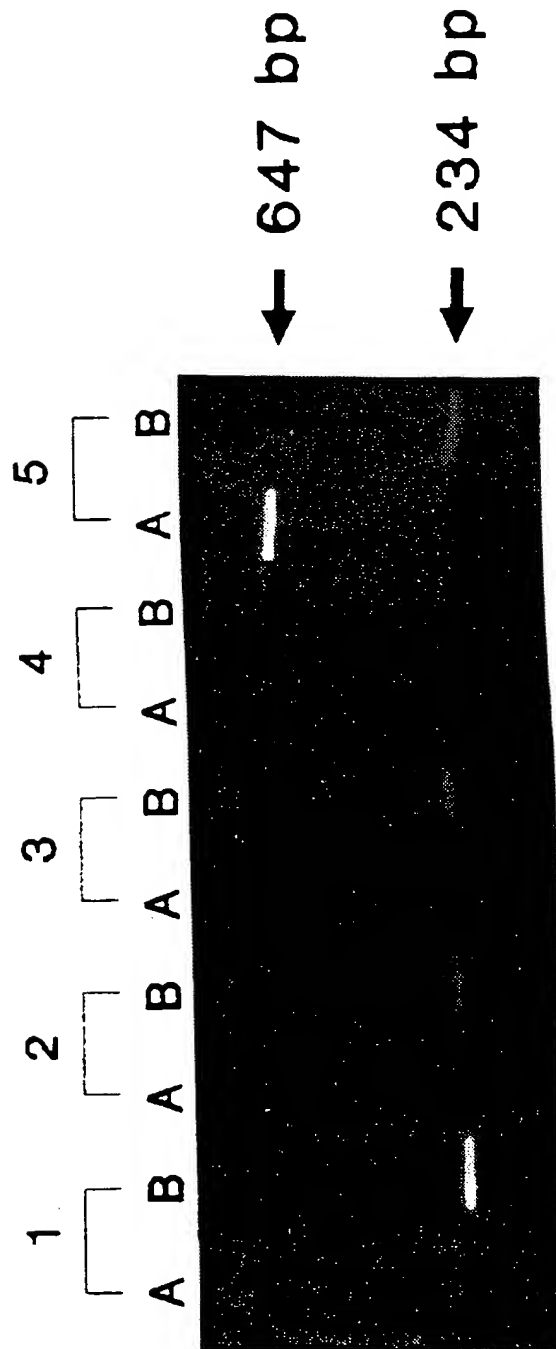


FIGURE 24



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FIGURE 26

♂ ♀ M H 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

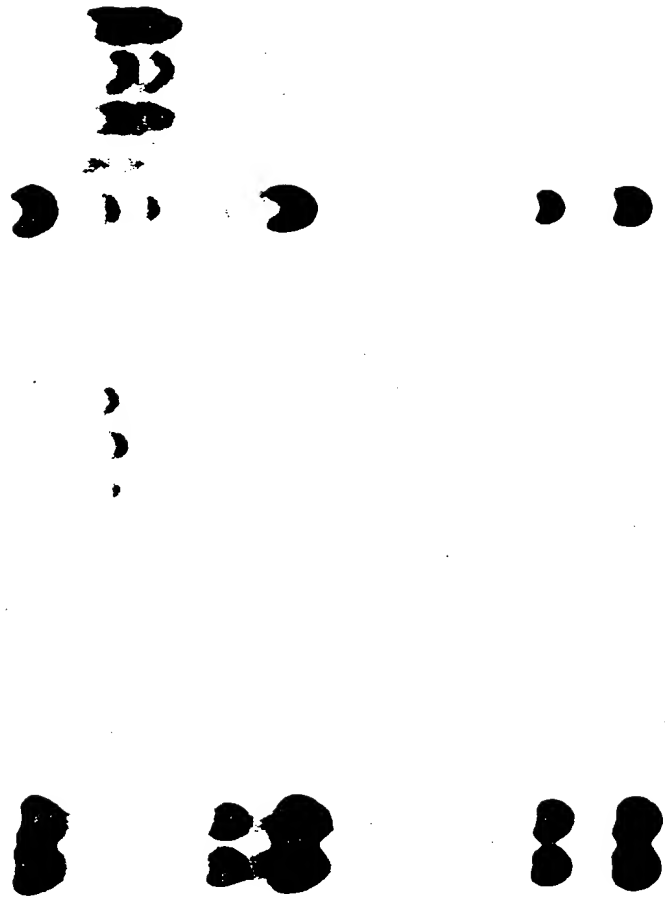


FIGURE 25

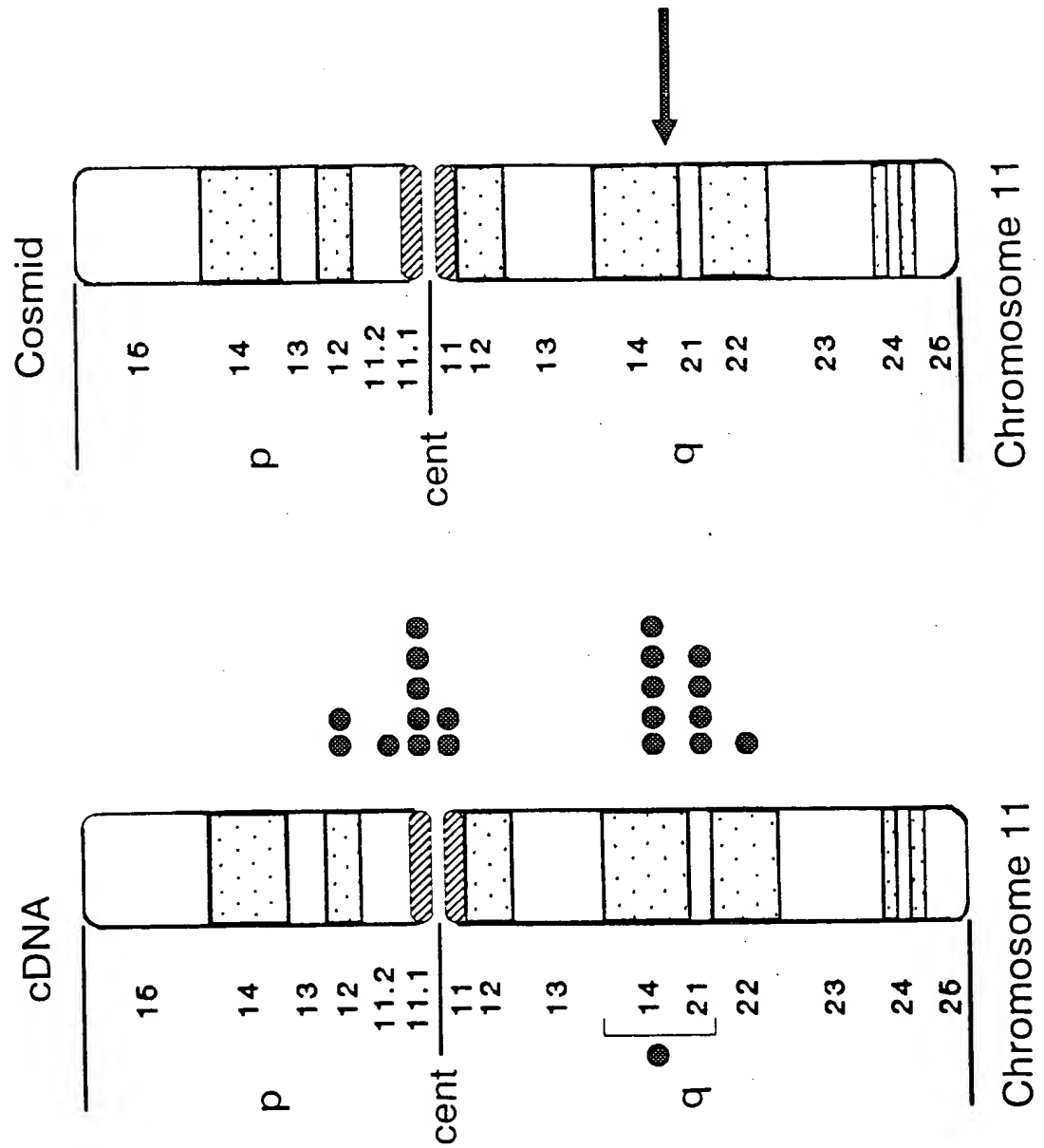
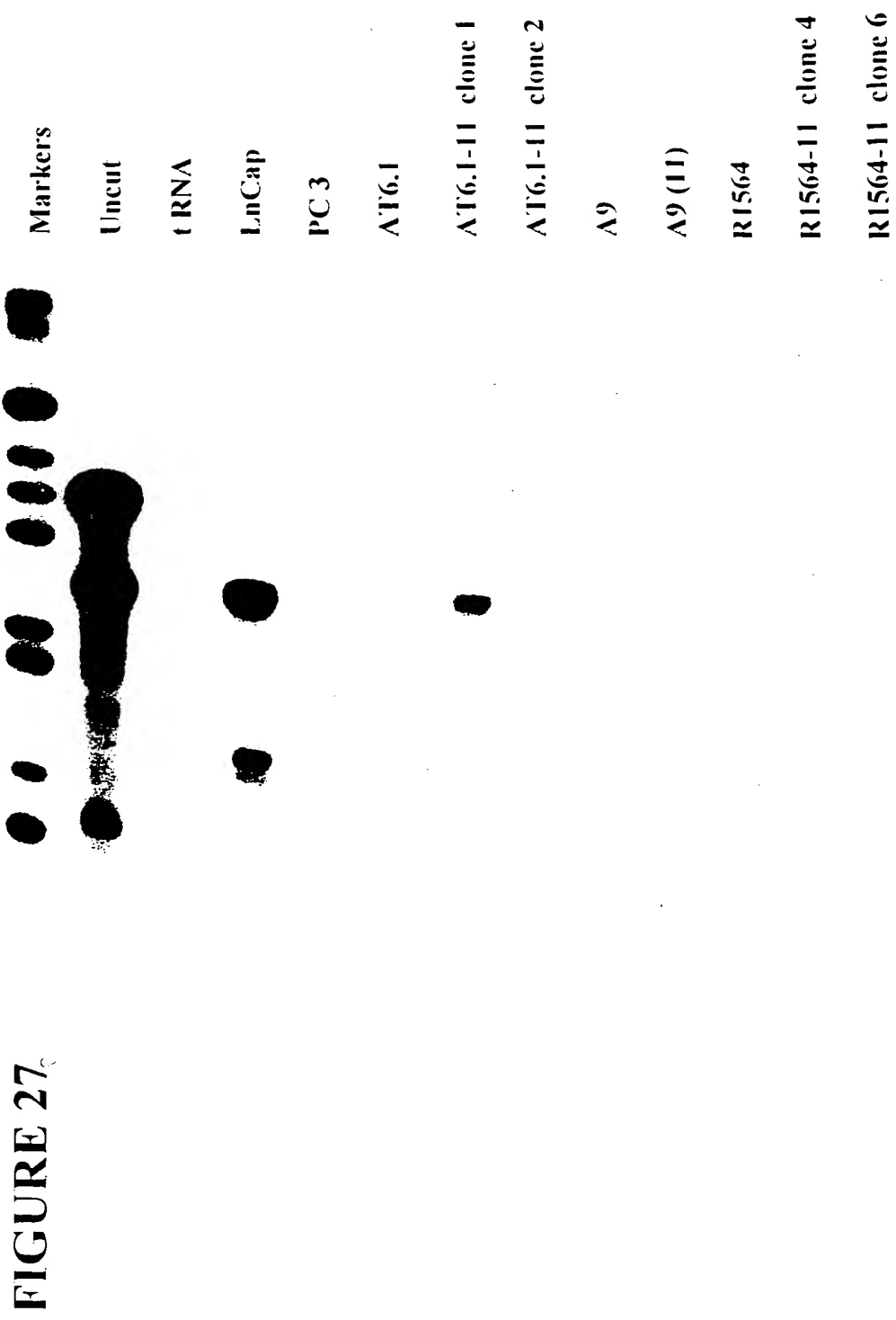


FIGURE 28

TISSUE/ CELL LINE	CANCER CELL TYPE	¹ PSM DNA	² PSM RNA
HUMAN PROSTATE	N.A.	+	+
HUMAN MAMMARY	N.A.	+	-
AT6.1	RAT PROSTATIC ADENOCARCINOMA	-	-
AT6.1-11-CL1	"	+	+
AT6.1-11-CL2	"	-	-
R1564	RAT MAMMARY ADENOCARCINOMA	-	-
R1564-11-CL2	"	+	-
R1564-11-CL4	"	+	-
R1564-11-CL5	"	+	-
R1564-11-CL6	"	+	-
A9	MOUSE FIBROSARCOMA	-	-
A9(11)	"	+	-



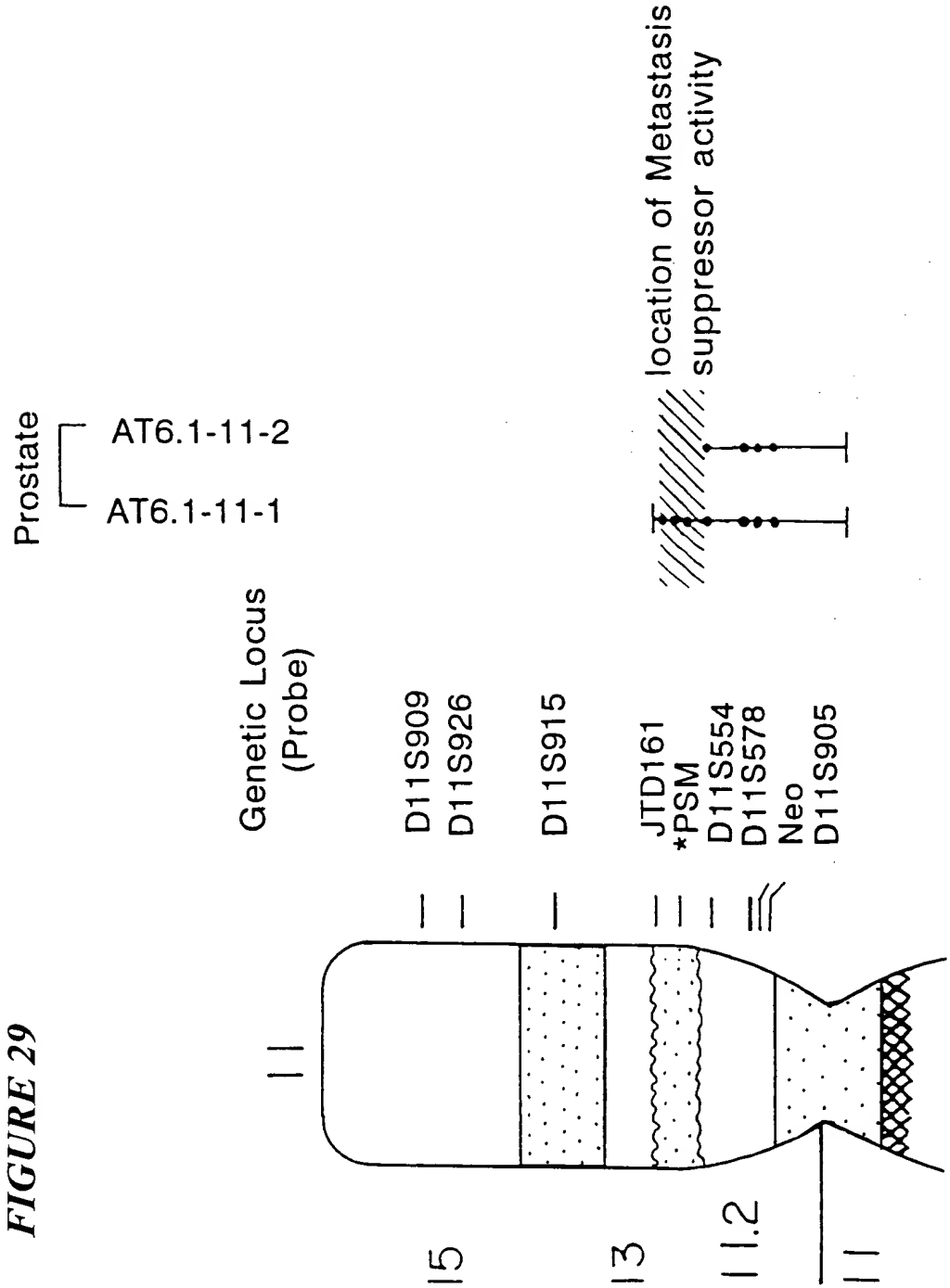


FIGURE 30

FIG. 55

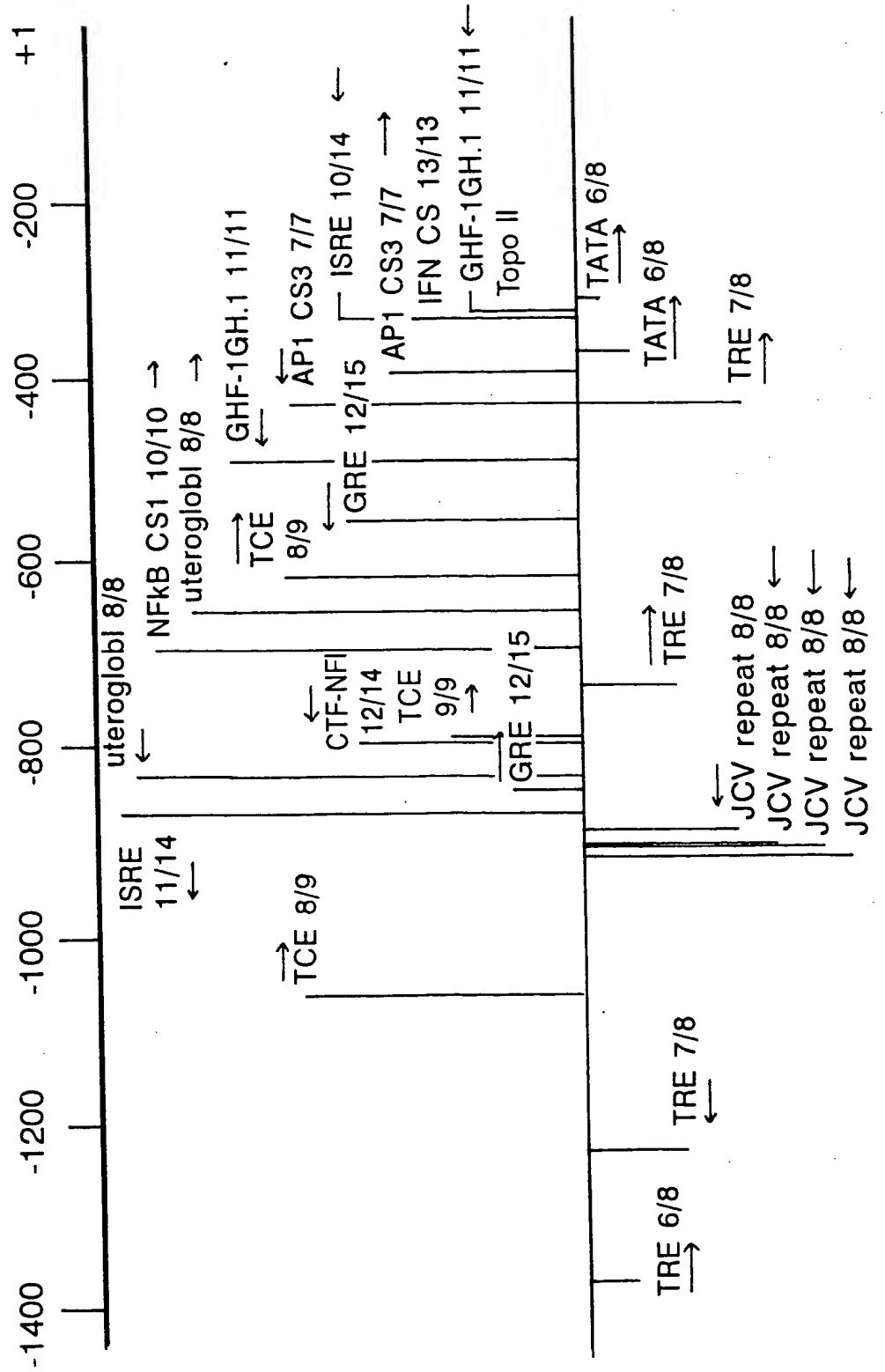


FIGURE 31

Prostate Specific Promoter: Cytosine Deaminase Chimera

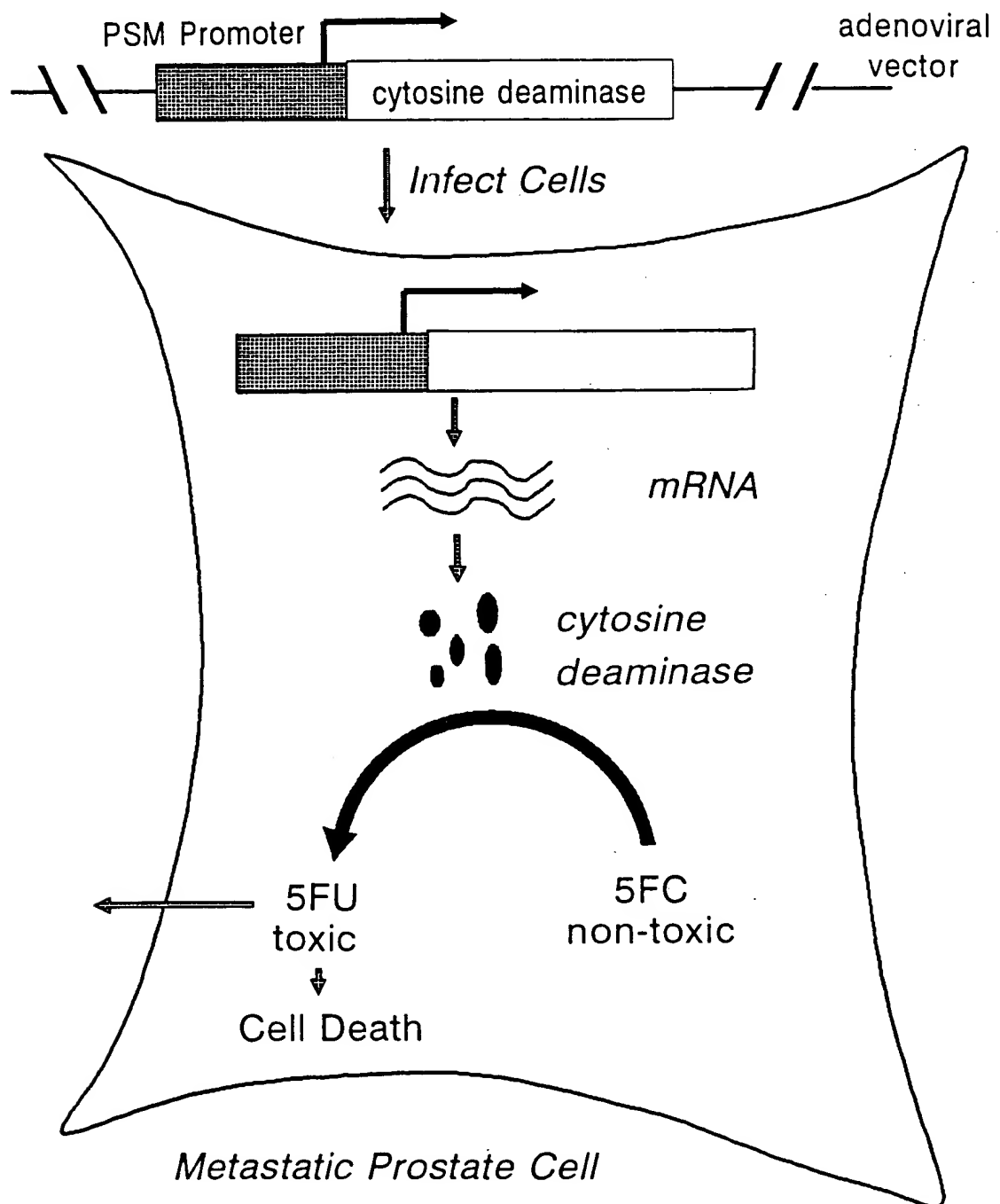


FIGURE 32A

	10	20	30	40	50	60
1	AAGGGTGCTC TTCCACGAG	CTTAGGCTGA GAATCCGACT	ATGCTTGCAG TACGAACGTC	ACAGGATGCT TGTCCCTACGA	TGGTTACAGA ACCAATGTCT	TGGGCTGTGA ACCCGACACT
61	CTCGAGTGGA GAGCTCACCT	GTTTTATAAG CAAAATATTG	GGTGCTCCTT CCACGAGGAA	AGGCTGAATG TCCGACTTAC	CTTGCAGACA GAACGTCTGT	GGATGCTTGG CCTACGAACC
121	TTACAGATGG AATGTCTACC	GCTGTGAGCT CGACACTCGA	GGGTGCTTGT CCCACGAACA	AAGAGGATGC TTCTCCTACG	TTGGGTGCTA AAGCCACGAT	AGTGAGCCAT TCACTCGGTA
181	TTGCAGTTGA AACGTCAACT	CCCTATTCTT GGGATAAGAA	GGAACATTCA CCTTGTAAGT	TTCCCTCTA AAGGGGAGAT	CCCCTGTTTC GGGGACAAAG	TGTTCTTGCC ACAAGGACGG
241	AGCTAAGCCC TCGATTCCGG	ATTTTTTCATT TAAAAAGTAA	TTTCTTTTAA AAAGAAAATT	CTCCTTAGCG GAGGAATCGC	CTCCGCAAAA GAGGCGTTTT	CTTAATCAAT GAATTAGTTA
301	TTCTTTAAAC AAGAAATTTG	CTCAGTTTTTC GAGTCAAAAG	TTATCTGTAA AATAGACATT	AAGGTAAATA TTCCATTTAT	ATAATACAGG TATTATGTCC	GTGCAACAGA CACGTTGTCT
361	AAAATCTAGT TTTTAGATCA	GTGGTTTACA CACCAAATGT	TAATCACCTG ATTAGTGGAC	TTAGAGATTT AATCTCTAAA	TAAATTATTT ATTTAATAAA	CAGGATAAGT GTCCTATTCA
421	CATGATAATT GTACTATTAA	AAATGAAATA TTACTTTTAT	ATGCACATAA TACGTGTATT	AGCACATAGT TCGTGTATCA	GTGGTGTCTT CACCACAGGA	CCATATAGAA GGTATATCTT
481	AATGCTCAGT TTACGAGTCA	ATATTGGTTA TATAACCAAT	TTAACTACTT AATTGATGAA	GTTGAAGGTT CAACTTCCAA	TATCTTCTCC ATAGAAGAGG	ACTAAACTGT TGATTTGACA
541	AAGTTCCACA TTCAAGGTGT	AGCCTTACAA TCGGAATGTT	TATGTGACAG ATACACTGTC	ATATTCATTG TATAAGTAAG	ATTGTCTGAA TAACAGACTT	TTCTTCAAAT AAGAAGTTTA
601	ACATCCTCTT TGTAGGAGAA	CACCATAGCG GTGGTATCGC	TCTTATTAAT AGAATAATTA	TGAATTATTA ACTTAATAAT	ATTGAATAAA TAACTTATTT	TTCTATTGTT AAGATAACAA
661	CAAAAATCAC GTTTTTAGTG	TTTTATATTT AAAATATAAA	AACTGAAATT TTGACTTTAA	TGCTTACTTA ACGAATGAAT	TAATCACATC ATTAGTGTAG	TAACCTTCAA ATTGGAAGTT
721	AGAAAACACA TCTTTTGTGT	TTAACCAACT AATTGGTTGA	GTAAGGGGTA CATGACCCAT	ATGTTACTGG TACAATGACC	GTGATCCAC CACTAGGGTG	GTTTTACAAA CAAAATGTTT

FIGURE 32B

781 TGAGAAGATA TATTCTGGTA AGTTGAATAC TTAGCACCCA GGGGTAATCA GCTTGGACAG
 ACTCTTCTAT ATAAGACCAT TCAACTTATG AATCGTGGGT CCCCATTAGT CGAACCTGTC

841 GACCAGGTCC AAAGACTGTT AAGAGTCTTC TGA CTCTCCAAA CTCAGTGCTC CCTCCAGTGC
 CTGGTCCAGG TTTCTGACAA TTCTCAGAAG ACTGAGGTTT GAGTCACGAG GGAGGTCACG

901 CACAAGCAAA CTCCATAAAG GTATCCTGTG CTGAATAGAG ACTGTAGAGT GGTACAAAGT
 TGTTTCGTTT GAGGTATTTT CATAGGACAC GACTTATCTC TGACATCTCA CCATGTTTCA

961 AAGACAGACA TTATATTAAG TCTTAGCTTT GTGACTTCGA ATGACTTACC TAATCTAGCT
 TTCTGTCTGT AATATAATTC AGAATCGAAA CACTGAAGCT TACTGAATGG ATTAGATCGA

1021 AAATTTTCAGT TTTACCATGT GTAAATCAGG AAGAGTAATA GAACAAACCT TGAAGGGTCC
 TTTAAAGTCA AAATGGTACA CATTTAGTCC TTCTCATTAT CTGTTTGGGA ACTTCCCAGG

1081 CAATGGTGAT TAAATGAGGT GATGTACATA ACATGCATCA CTCATAATAA GTGCTCTTTA
 GTTACCACTA ATTTACTCCA CTACATGTAT TGTACGTAGT GAGTATTATT CACGAGAAAT

1141 AATATTAGTC ACTATTATTA GCCATCTCTG ATTAGATTTG ACAATAGGAA CATTAGGAAA
 TTATAATCAG TGATAATAAT CGGTAGAGAC TAATCTAAAC TGTTATCCTT GTAATCCTTT

1201 GATATAGTAC ATTCAGGATT TTGTTAGAAA GAGATGAAGA AATTCCTTTC CTTCTGCCC
 CTATATCATG TAAGTCCTAA AACAATCTTT CTCTACTTCT TTAAGGGAAG GAAGGACGGG

1261 TAGGTCATCT AGGAGTTGTC ATGGTTCATT GTTGACAAAT TAATTTTCCC AAATTTTTC
 ATCCAGTAGA TCCTCAACAG TACCAAGTAA CAACTGTTTA ATTAAGGGAAG TTTAAAAAGT

1321 CTTTGCTCAG AAAGTCTACA TCGAAGCACC CAAGACTGTA CAATCTAGTC CATCTTTTTT
 GAAACGAGTC TTTCAGATGT AGCTTCGTGG GTTCTGACAT GTTAGATCAG GTAGAAAAAG

1381 CACTTAACTC ATACTGTGCT CTCCCTTTCT CAAAGCAAAC TGTTTGCTAT TCCTTGAATA
 GTGAATTGAG TATGACACGA GAGGGAAAAG GTTTCGTTT ACACGATA AGGAACTTAT

1441 CACTCTGAGT TTTCTGCCTT TGCCTACTCA GCTGGCCCAT GGCCCTAAT GTTCTTCTC
 GTGAGACTCA AAAGACGGAA ACGGATGAGT CGACCGGTA CCGGGGATTA CAAAGAAGAG

1501 ATCTCCACTG GGTCAAATCC TACCTGTACC TTATGGTTCT GTTAAAAGCA GTGCTTCCAT
 TAGAGGTGAC CCAGTTTAGG ATGGACATGG AATACCAAGA CAATTTTCGT CACGAAGGTA

1561 AAAGTACTCC TAGCAAATGC ACGGCCTCTC TCACGGATTA TAAGAACACA GTTTATTTTA

FIGURE 32C

TTTCATGAGG ATCGTTTACG TGCCGGAGAG AGTGCCTAAT ATTCTTGTGT CAAATAAAAT

1621 TAAAGCATGT AGCTATTCTC TCCCTCGAAA TACGATTATT ATTATTAAGA ATTTATAGCA
ATTTTCGTACA TCGATAAGAG AGGGAGCTTT ATGCTAATAA TAATAATTCT TAAATATCGT

1681 GGGATATAAT TTTGTATGAT GATTCTTCTG GTTAATCCAA CCAAGATTGA TTTTATATCT
CCCTATATTA AAACATACTA CTAAGAAGAC CAATTAGGTT GGTTCTAACT AAAATATAGA

1741 ATTACGTAAG ACAGTAGCCA GACATAGCCG GGATATGAAA ATAAAGTCTC TGCCTTCAAC
TAATGCATTG TGTATCGGT CTGTATCGGC CCTATACTTT TATTTTCAGAG ACGGAAGTTG

1801 AAGTTCCAGT ATTCTTTTCT TTCCTCCCCT CCCCTCCCCT CCCTTCCCCT CCCCTTCCTT
TTCAAGGTCA TAAGAAAAGA AAGGAGGGGA GGGGAGGGGA GGGGAAGGGGA GGGGAAGGAA

1861 CCCTTTCCCCT TCCCTTCCTT TCTTTCTTGA GGGAGTCTCA CTCTGTCACC AGGCTCCAGT
GGGAAAGGGA AGGGAAGGAA AGAAAGAACT CCCTCAGAGT GAGACAGTGG TCCGAGGTCA

1921 GCAGTGGCGC TATCTTGGCT GACTGCAACC TCCGCTCCC CGGTTCAAGC GATTCTCCTG
CGTCACCGCG ATAGAACCGA CTGACGTTGG AGGCGGAGGG GCCAAGTTCT CTAAGAGGAC

1981 CCTCAGCCTC CTGAGTAGCT GGGACTACAG GAGCCCGCCA CCACGCCCAG CTAATTTTTG
GGAGTCGGAG GACTCATCGA CCCTGATGTC CTCGGGCGGT GGTGCGGGTC GATTAAAAAC

2041 TATTTTTAGT AGAGATGGGG TTTCACCATG TTGGCCAGGA TGGTCTCGAT TTCTCGACTT
ATAAAAATCA TCTCTACCCC AAAGTGGTAC AACCGGTCCT ACCAGAGCTA AAGAGCTGAA

2101 CGTGATCCGC CTGTCTGGGC CTCCCAAAGT GCTGGGATTA CAGGCGTGAG CCACCACGCC
GCACTAGGCG GACAGACCCG GAGGGTTTCA CGACCCTAAT GTCCGCACTC GGTGGTGCGG

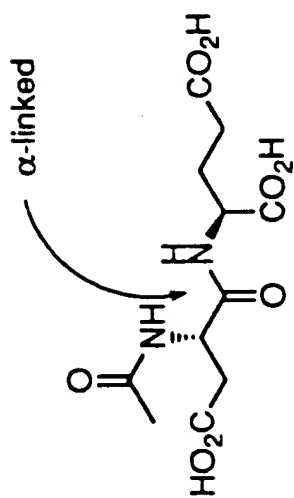
2161 CGGCTTTAAA AAATGGTTTT GTAATGTAAG TGGAGGATAA TACCCTACAT GTTTATTAAT
GCCGAAATTT TTTACCAAAA CATTACATTC ACCTCCTATT ATGGGATGTA CAAATAATTA

2221 AACATAATA TTCTTTAGGA AAAAGGGCGC GGTGGTGATT TACACTGATG ACAAGCATTC
TTGTTATTAT AAGAAATCCT TTTCCCGCG CCACCACTAA ATGTGACTAC TGTTTCGTAAG

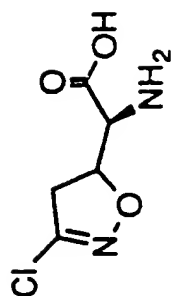
2281 CCGACTATGG AAAAAAAGCG CAGCTTTTTT TGCTCTGCTT TTATTCAGTA GAGTATTGTA
GGCTGATACC TTTTTTTCGC GTCGAAAAAG ACGAGACGAA AATAAGTCAT CTCATAACAT

2341 GAGATTGTAT AGAATTTTCA AGTTGAATAA AAGTTCCTCA TAATTATAGG AGTGGAGAGA
CTCTAACATA TCTTAAAGTC TCAACTTATT TTCAAGGAGT ATTAATATCC TCACCTCTCT

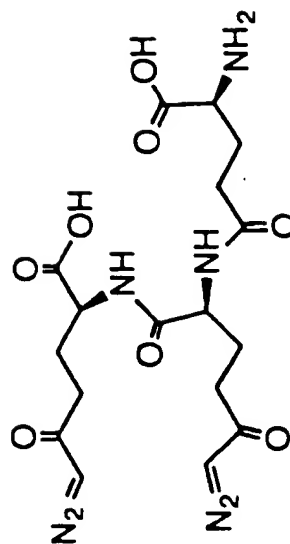
FIGURE 33



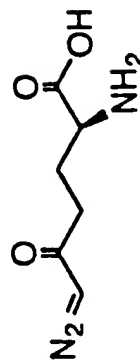
NAAG 1
N-acetylaspartyl-L-glutamate



Acivicin

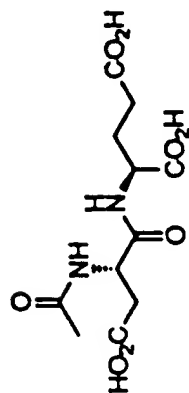


Azotomycin, becomes active by *in vivo* conversion to DON

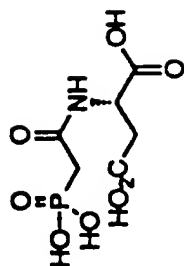


6-diazo-5-oxo-norleucine, DON

FIGURE 34



NAAG 1
N-acetyl-L-glutamate



PALA 2
N-phosphonoacetyl-L-aspartate



D-APV
L-Glu antagonist

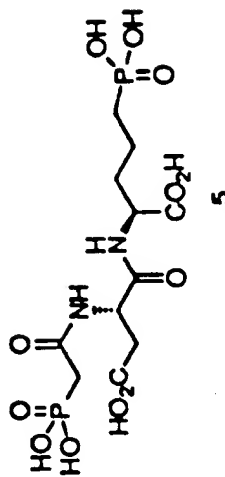
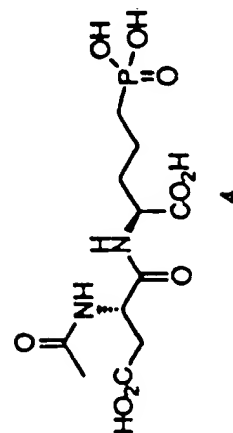
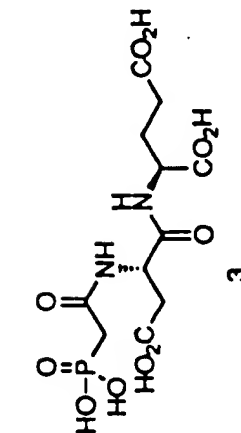
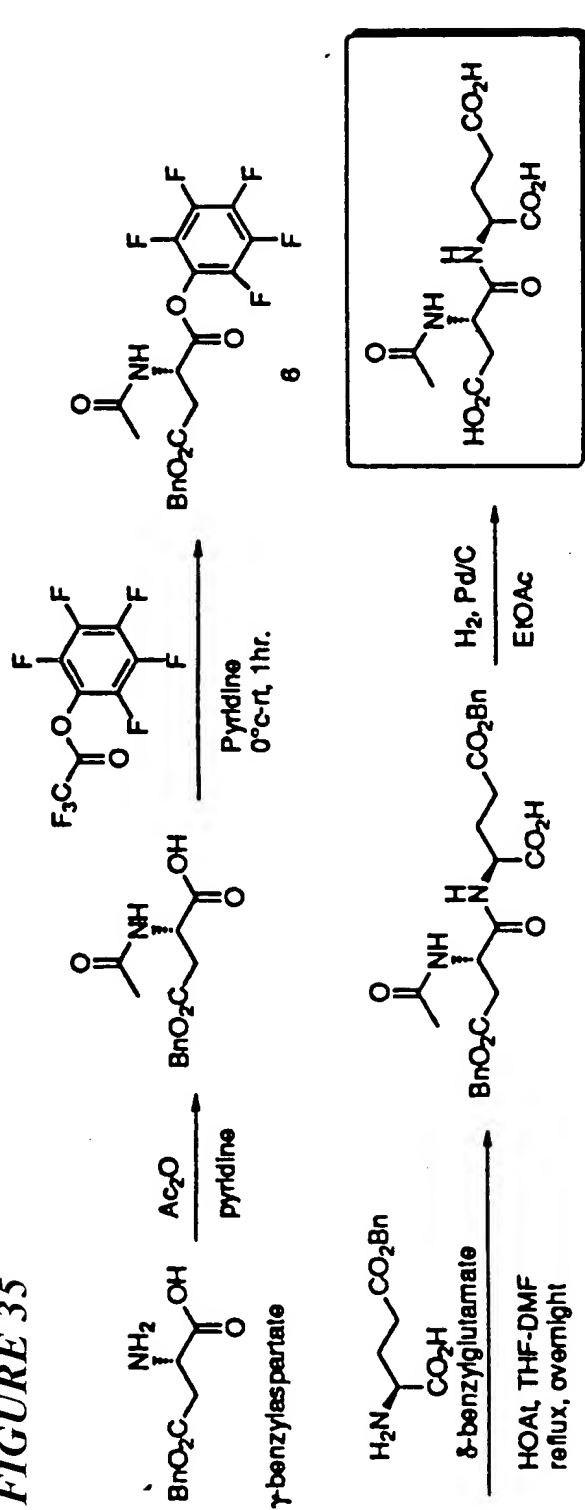
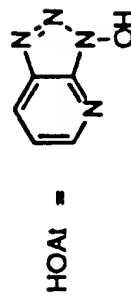


FIGURE 35



1
 NAAG
 Identical in all respects to an authentic sample from Sigma.

Ac₂O = acetic anhydride
 THF = tetrahydrofuran
 DMF = N,N-dimethylformamide
 Pd/C = palladium on charcoal
 EtOAc = ethylacetate



$$\begin{array}{c}
 \text{EtO} \quad \text{EtO} \\
 \diagup \quad \diagdown \\
 \text{P} \\
 \parallel \\
 \text{O} \\
 \text{EtO} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{C}(=\text{O}) \text{---} \text{OC}_6\text{F}_5
 \end{array}
 \xrightarrow[\text{THF, reflux}]{\text{HOAt}}
 \begin{array}{c}
 \text{NH}_2 \\
 | \\
 \text{BnO}_2\text{C} \text{---} \text{CH}_2 \text{---} \text{CH}(\text{OH}) \text{---} \text{C}(=\text{O})
 \end{array}
 \quad \text{7}$$

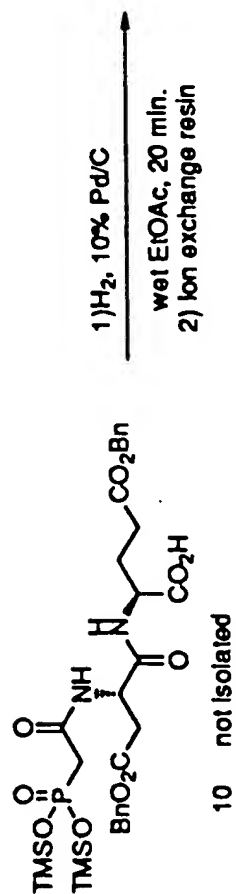
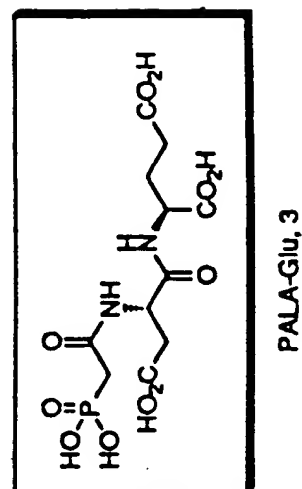
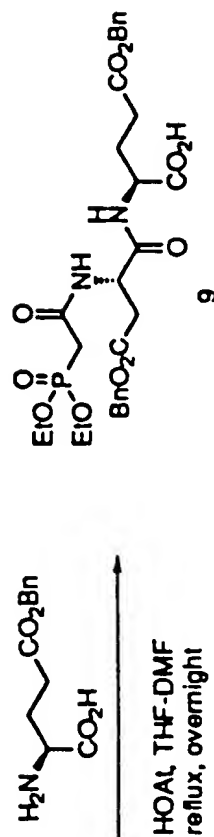
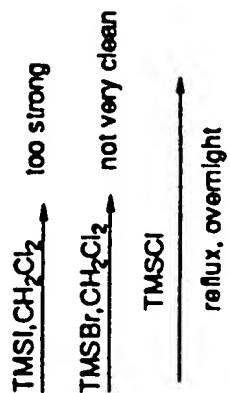
$$\begin{array}{c}
 \text{EtO} \quad \text{EtO} \\
 \diagup \quad \diagdown \\
 \text{P} \\
 \parallel \\
 \text{O} \\
 \text{EtO} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{C}(=\text{O}) \text{---} \text{OC}_6\text{F}_5
 \end{array}
 \xrightarrow[\text{Py}]{\text{CF}_3\text{CO}_2\text{C}_6\text{F}_5}
 \begin{array}{c}
 \text{EtO} \quad \text{EtO} \\
 \diagup \quad \diagdown \\
 \text{P} \\
 \parallel \\
 \text{O} \\
 \text{EtO} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \text{---} \text{C}(=\text{O}) \text{---} \text{NH} \text{---} \text{CH}(\text{OC}_6\text{F}_5) \text{---} \text{CH}_2 \text{---} \text{C}(=\text{O}) \text{---} \text{OC}_6\text{F}_5
 \end{array}
 \quad \text{8}$$


FIGURE 37

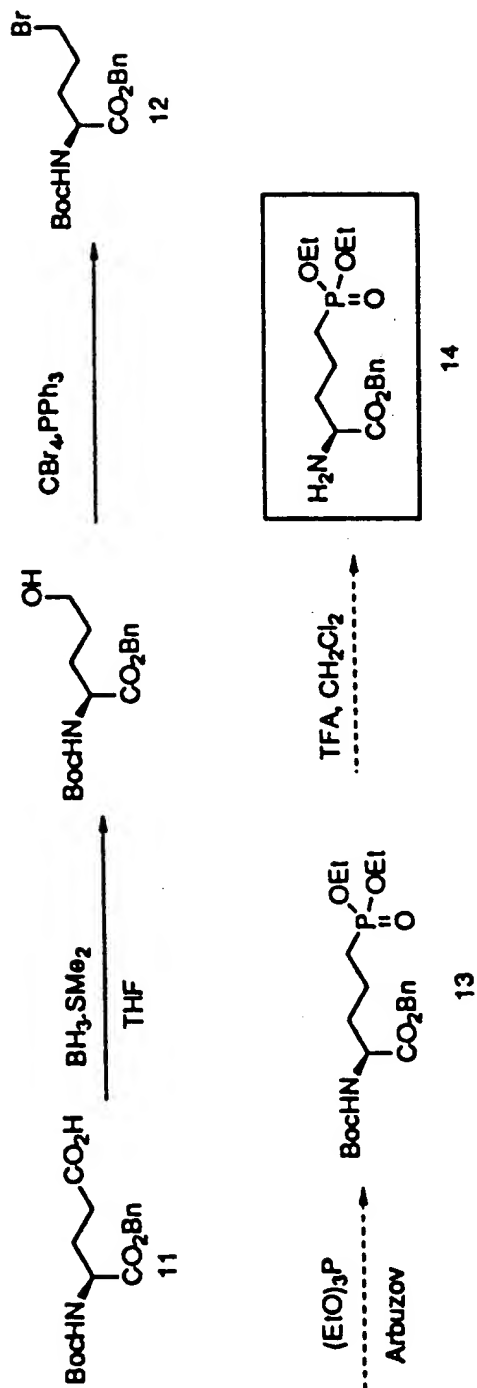


FIGURE 38

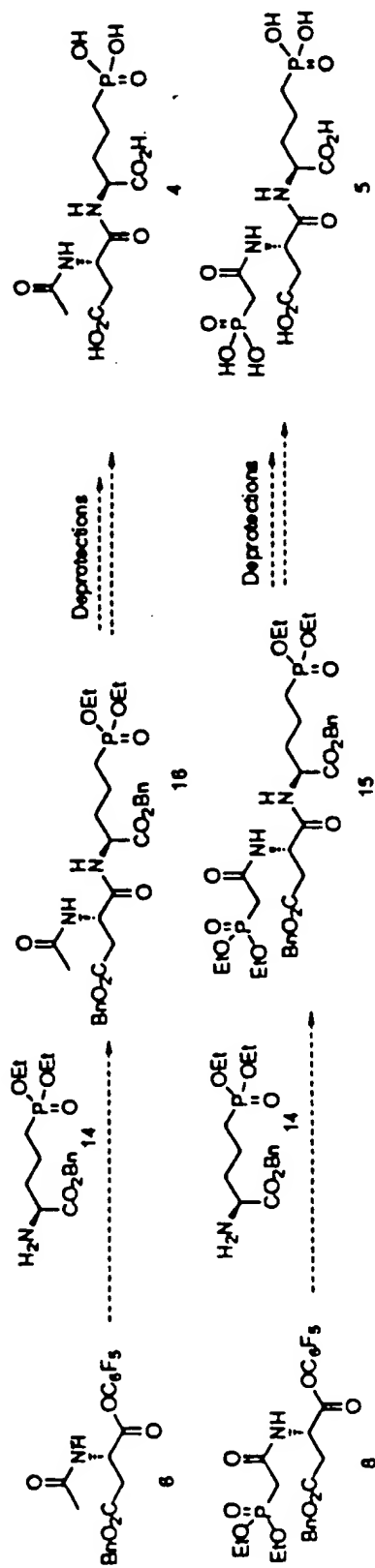
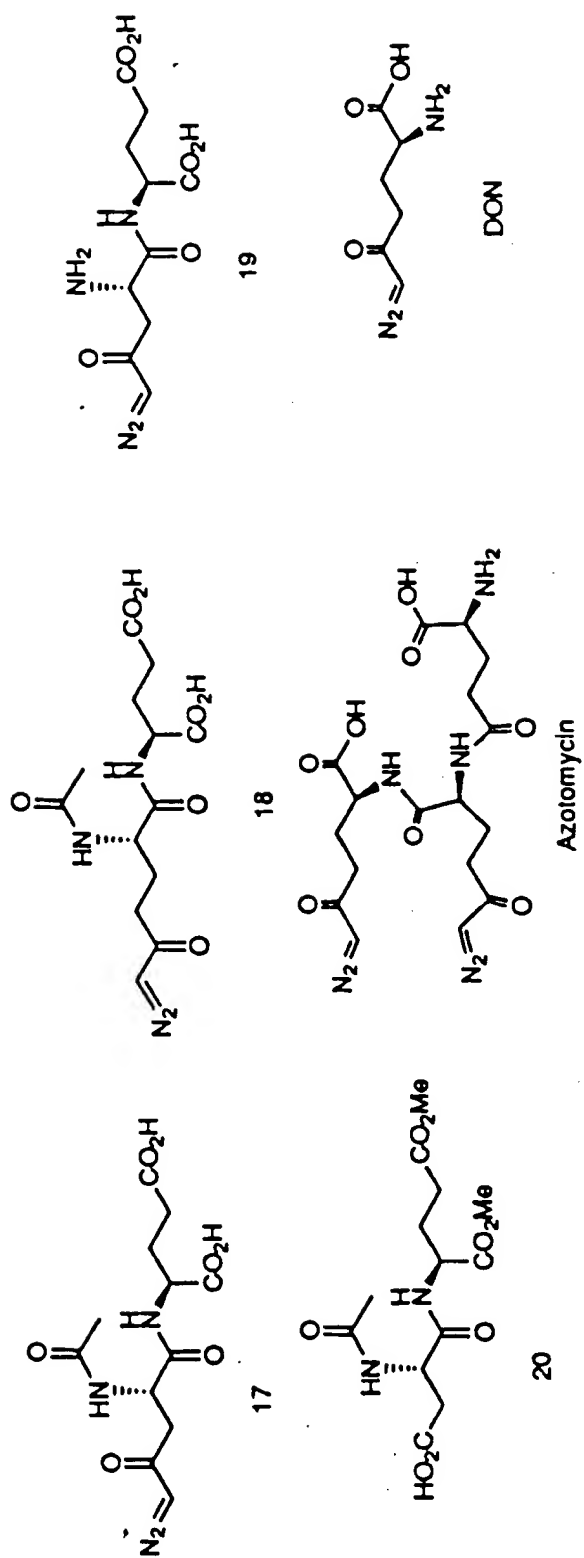
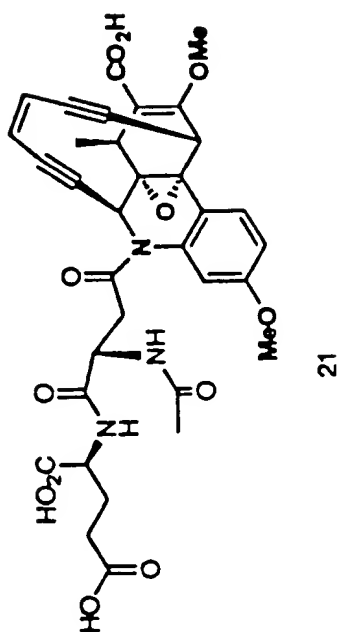
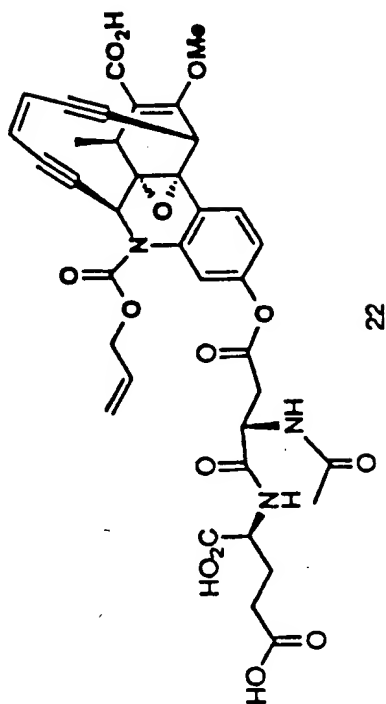


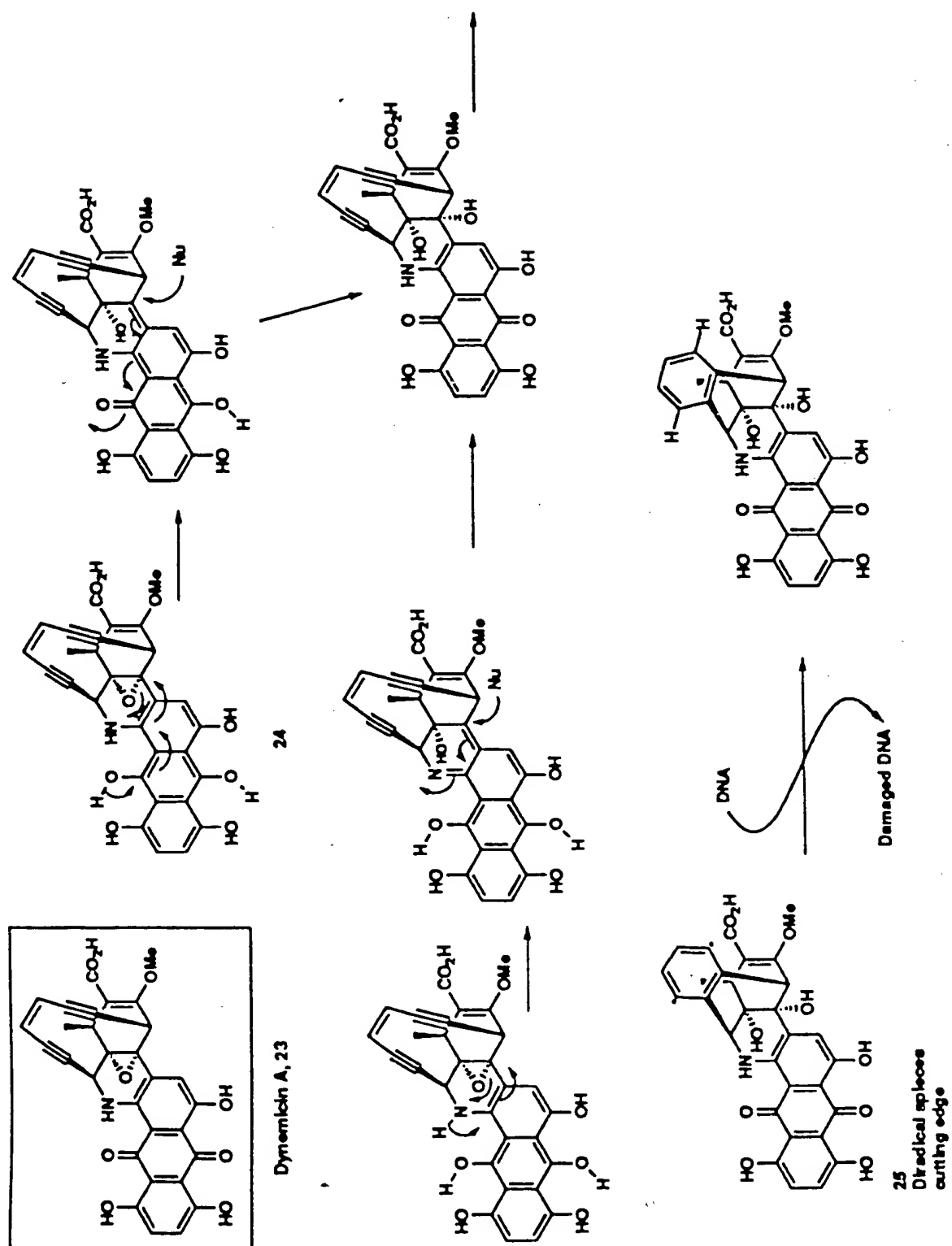
FIGURE 39



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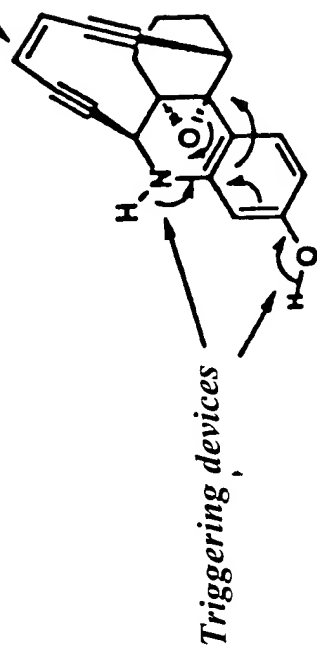
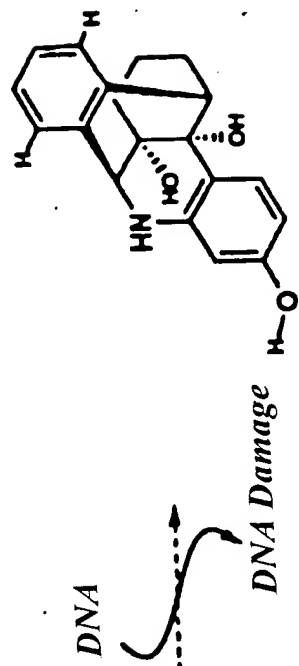
FIGURE 40





Warhead

FIGURE 42



26

active at the nano to picomolar levels in different cell lines readily rearranges when one or both triggering devices are deprotected

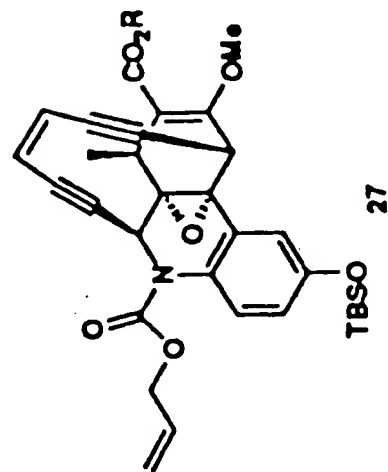
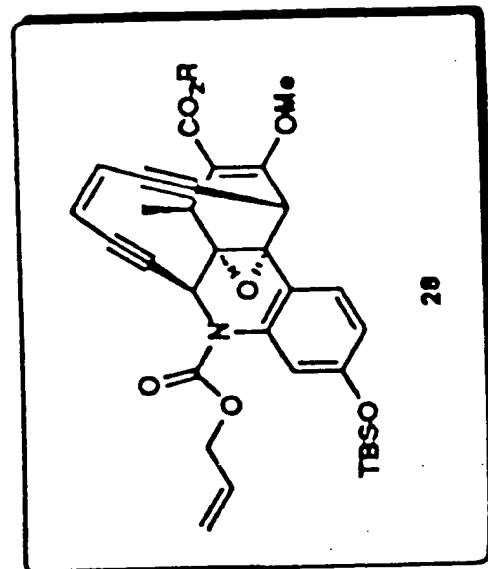
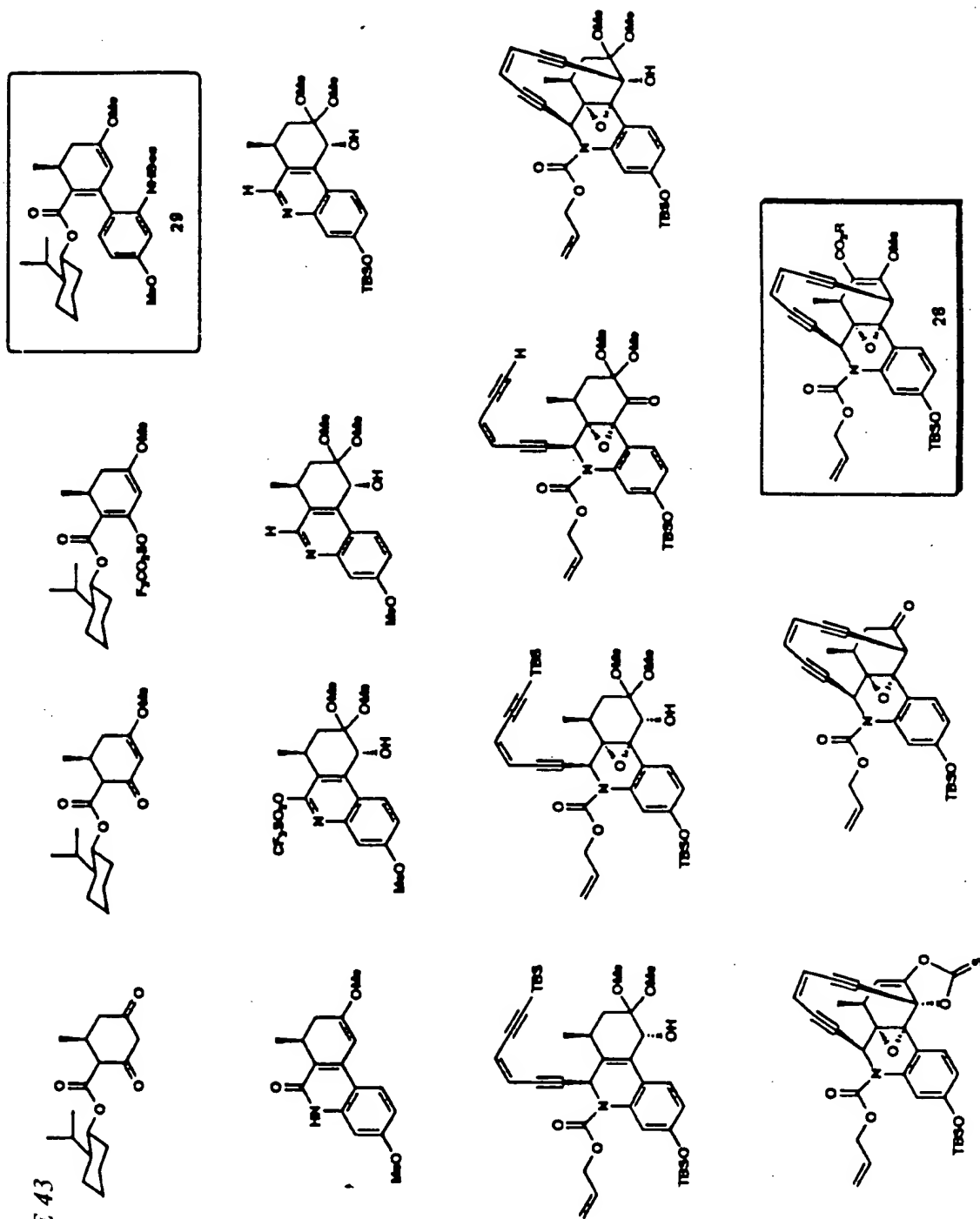


FIGURE 43



17 Steps for the optically active form 1

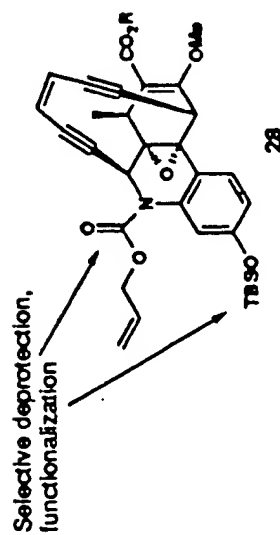
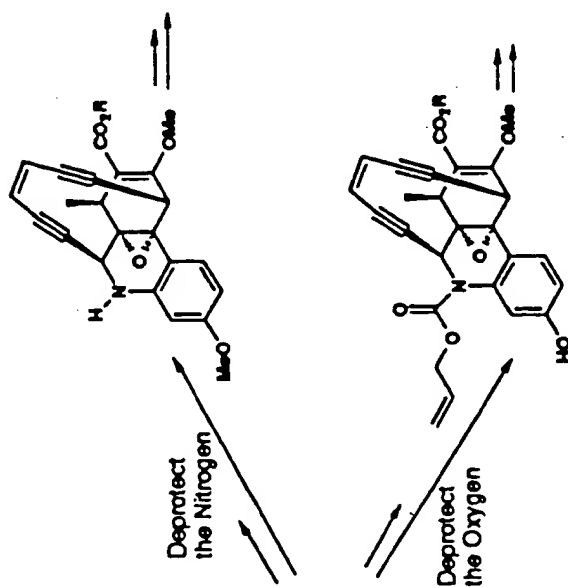
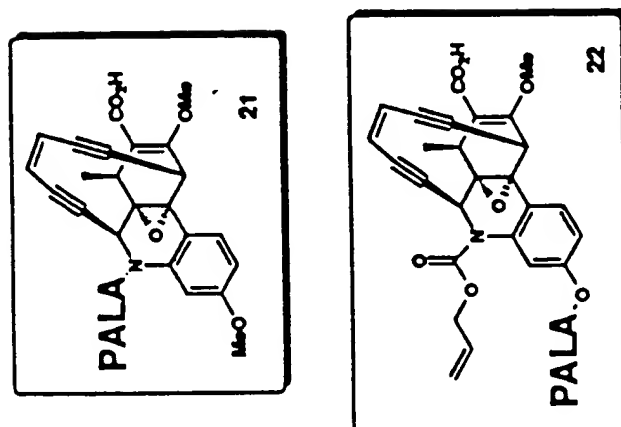


FIGURE 44

FIGURE 45

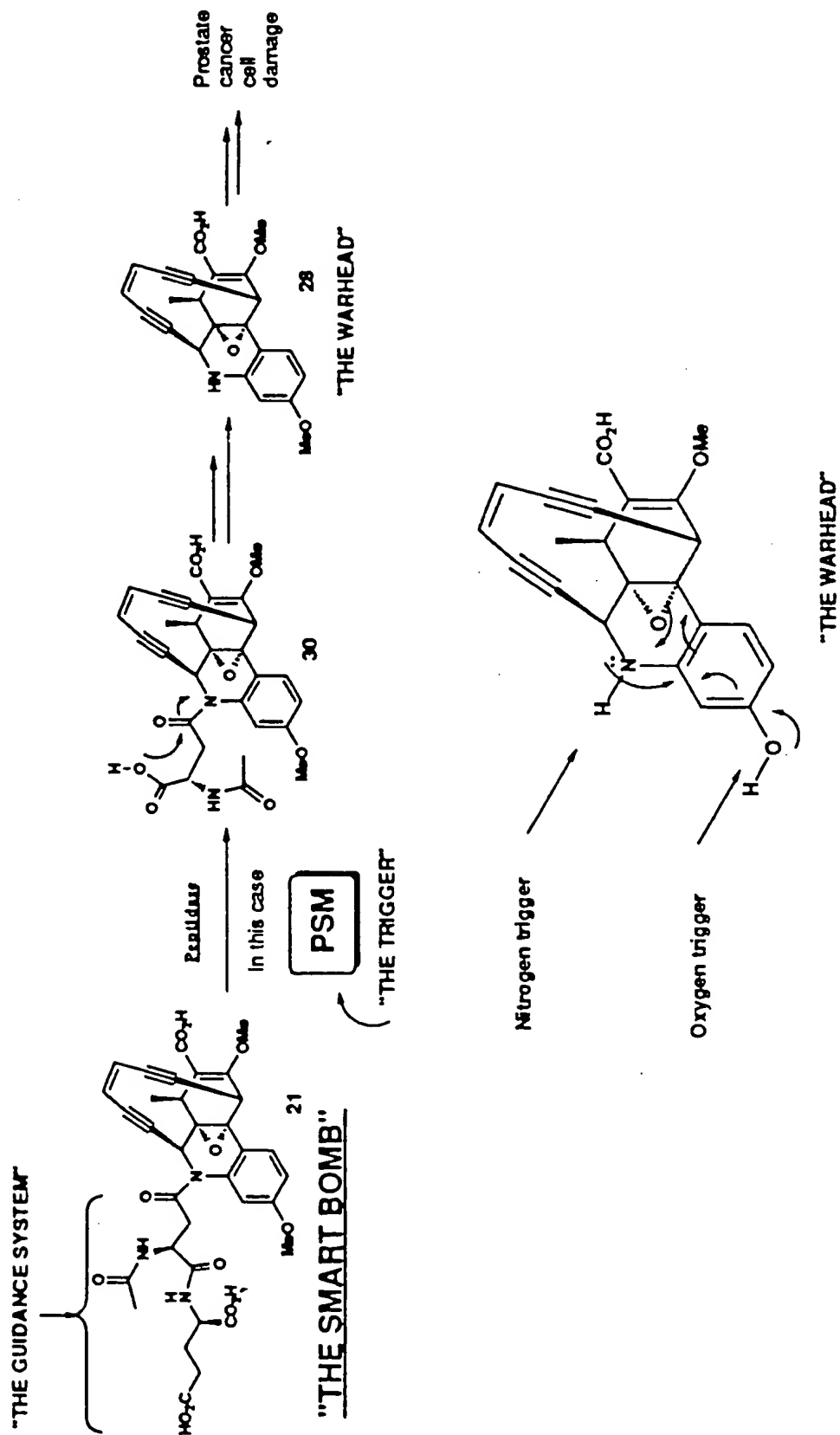


FIGURE 46A

	10	20	30	40	50	60
1	TAGGGGGGGG NTCCCCCGC	CCTCGGGAG GGAGCGCCTC	AAACCTCGGA TTTGGAGCCT	GICTTCCCG CAGAAGGGB	TGGTGCCGC ACCACGGCG	GTGCTGGGAC CACGACCCCTG
61	TCGCGGGTCA	GCTGCCGAGT CGACGGCTCA	GGGATCCTGT CCCTAGGACA	TGCTGGTCTT ACGACCAGAA	CCCCAGGGGC GGGTCCCCG	GGCGATTAGG CCGCTAATCC
121	GTCGGGGTAA	TGTGGGGTGA ACACCCCACT	GCACCCCTCG CGTGGGAGC	ACTTAGGAGG TCAATCCTCC	AGGTAGCTG TCCCATCGAC	GGAAACGGTGC CCTTGCCACG
181	AGGGCTGAGT TCCCGACTCA	TCTC3ACAAG AGAGCTGTTC	CTGCTGGTAG GACGACCATC	GACAGTCACT CTGTCAGTGA	CAGGTTGAGG GTCCAACTCC	GTAGAACTGA CATCTTGACT
241	GAGAACTGA CTCTTGACT	AAC TGCGCGT TTGACCCGCA	AGGAAGGTTT TCCTTCCAAG	CAAGTGCTGG GTTACGAGCC	AGCCCTGCCA TCGGGACGTT	GACAGAGGAA CTGTCTCCTT
301	GTTTTTTTTT CAAAAAAAA	TGCTTTTGT ACGAACAA	TTGTTTGT AACAAACAA	TTGTTTGT AACAAACAA	TTGTTTGT AACAAACAA	TGTTTGT ACAAACAA
361	TTTTTTTACC AAAAAATGG	TCTCTGTGCA AGAGACACGT	TTCTTTCTTC AAGAAAGAAG	CTTGGAAAGTA GAACCTTCAT	ACAGAGGCCA TGCTCCTCGT	GCTTGGGAAC CGAACCCCTTG
421	TGTGTGAACC ACACACTTGG	AGGTCAGCAA TCCAGTCGTT	TC TGGACAGG AGACCTGTCC	TC TTTACCAG AGAAATGGTC	CGGTCCTTTT GCCCAGAAJA	GCTGTTTTTC CGACAAAAAG
481	CTGGGTA CTG GACCCATGAC	ATTTGCAGAC TAAACGTCTG	TTGATCCAAAC AACTAGGTTG	TTTCTAAGAA AAAGATTCTT	AAGCAGAACC TTCGTCTTGG	ACACAGGCCAA TGTTGTCGGT
541	GCTCAGACTC CGAGTCTGAG	TTTTATTAAA AAAAATAATT	TTCCAGTTT AAGGTCAAAA	GACTTTGCCA CTGAACCGT	CTTCTTAGTG GNAGAAATCAC	GCCTTGAAACA CGGAACCTTGT

FIGURE 46B

301 AGTTACCGAG TCCCTCTCAG CGTTAGTTAC CCTATTTTAT GATGAGGATA ATATTATCTG
 TCAATGGCTC AGGAGAGTC GCAATCAATG GGATAAAATA CTACTCCTAT TATAATAGAC

 661 CAATATTATG GTAATAGTAA ATAATATAGC ATGTAAATCT CCIAGCACAG TACTGGGATT
 GTTTAATAAC CATTATCATT TATTATATCG TACATTTAGA GGATCGTGTC ATGACCCCTAA

 721 TTCGCCACTT TATTCTTCT TTTACCAAGA TACTCCTCAT TGGACTTTAA TACACAGGAC
 AAGCGGTGAA ATAAAGAAGA AATGGTTCT ATGAGGAGTA ACCTGAAATT ATGTGTCCTG

 781 TAGTCTAAGG TATCACCAGG TAGTCCACTC CTGCTCGGAA TTCTTGACCC TCTTTCGGGA
 ATCAGATICC ATAGTGTCC ATCAGGTGAG GACGAGCCTT AAGAACTGGG AGAAAGCCCT

 841 TTTAGAAGAA TAGGGCATGG ACCAGATGGG TTTAAACAAA TTCAATATCT TCCACTAGCT
 AATCTTCTT ATCCCGTACC TGGTCTACCC AATTGTGTTT AAGTTATAGA AGGTGATCGA

 901 TCACCTTGGG GTTGTTAAAA GATTTTGA A CCACACACTG TGCTCATAC AATCTTCATC
 AGTGGAAACC CAACAATTT CTAAAAACTT GGTGTGTGAC ACGAGTATTG TTAGAAGTAG

 961 TCTTAAAGG ATTTATTCT TCCTGGTATT GCCCTCACTC TCATCCCTGT ATTCCGTGCT
 AGAATTTTCC TAAAAATAAGA AGGACCATAA CGGGAGTGAG ACTAGGGACA TAAGGCACGA

1021 CAGTGGCTGA CACAGAAGAG TTCTTTATTG ATGTCGGCCC CCCACCCACT AGGATTCTCT
 GTCACCGACT GTGTCTTCTC AAGAAATAAC TACAGGCGGG GGTGGGTGA TCCTAAGAGA
 1081 GCTCTCCCT CCCCTACAG GCCTCCATCC TCTTCATCCT GTTCATTTT CAGATCTCAG
 CGAGAGGGA GGGGATGTC CGGAGGTAGG AGAAGTAGGA CAAGTAAAA GTCTAGAGTC
 1141 TTCAAGCATC TCGTCCCTCAG TGTGGTGTTC CCTGATCCCT CACTCTAATC CAAGTCTTTC
 AAGTTCOTAG AGCAGGAGTC ACACCAAAA GGAAGTAGGA GTGAGATTAG GTTCAGAAAG
 1201 TGTTTTATGC ACAGGTGGAA TCTTATTTC GTTGCGTCC AATCATGTAT TTTAATATGC
 ACAAATACG TGTCCACCTT AGAATAAAG CAAAGCAGG TTAGTACATA AAATTATACG
 1261 ATGTATATAT GTATGTGCAT TTGTATGCAT GCGATTAAAG ACTAGAATAA TTAATAATTG
 TACATATATA CATACACGTA AACATACGTA CGCTAATCT TGATCTTAT AATTATTAAAC
 1321 GAAAGCTCCA TGAAGCTGG TTGGGGACTA ATTTTGTAA TACTTTATTC CCAGATCCTG
 CTTTCGAGGT ACTTTCGACC AACCCCTGAT TAAACATTC ATGAATAAG GGTCTAGGAC
 1381 TAATTCTCT AAATAAACCC TGGAACTCTG CCTTATCTCC TTCAGGTTAA AAGCCAACTG
 ATTAAGAGA TTTATTGCG ACCTTAGAAC GGAATAGAGG AAGTCCAAT TTCCGTTGAC
 1441 CAAGGTCTAA TGACTGCAGG ATCTAGCTAT CCATTGTTTC TGGCCGCCCTA TGGTGCACT
 GTTCCAGATT ACTGACGTC TAGATCGATA GGTAAACAAAG ACGGCGGAT ACGCACGTGA
 1501 GGGTGTCTG CAGAGAGGCT GGGTAAATTG TAGTTTCATT GTAGCTGTCT GACTTGGATT
 CCCACAGACC GTCTCTCCGA CCCATTAAAC ATCAAGTAA CATCGACAGA CTGAACCTAA
 1561 TCTCAGCCT ACTTCACTGG AAACGCAAC TCTCACACCA TTTTGTTTTA GTTTCAGAAAT
 AGAGTGCGGA TGAAGTGACC TTTGCGTTTG AGAGTCTCGT AAACAAAAT CAAAGTCTTA
 1621 CAGAGCAAT TAGAAGTCTG AATTCCCTC AACACTTGA AATAATTAT TTATTGAAA
 GTCTCGTTTA ATCTTCAGAC TTAAGGAAG TTGTGAACCT TTATTAAATA AATAACTTT
 1681 TATATTCATA ATTAATTCGT TATAAAATG TATTAATGC TTATTGAGT CAGCAGAGGA
 ATATAAGTAT TAATTAGCA ATATTTTAC ATAATTACG AATAAACTCA GTCGTCTCCT

FIGURE 46C

FIGURE 46D

1741 AGATAGAAAC TTTATGAAAG TAGAAGGTGG ATCTCCTTTT TGCCTTCATT TTCAGAACAT
TCTATCTTTG AAATACTTC ATCTTCCACC TAGAGGAAA ACGAAGTAA AAGTCTTGTA

1801 CTCGTTTACA CCCATTAGTT GAACATTAA TGTCAATTTA TTTTCGTCCT GATTATCTCA
GAGCAAATGT GGGTAATCAA CTTTGTAATT ACAGTAAAT AAAAGCAGGA CTAATAGAGT

1861 TAAACATTT CTTAGAATAA CAGCAATACC TATCATTTGA GTTGGATAAG AAATATTTTG
ATTTTGTAAA GAATCTTATT GTCGTTATGG ATAGTAACTT CAACCTATTG TTTATATAAAC

1921 CAATTGGTTT GCAACTTAA AATCTGTTG CATGACTCTT TTTCAGTGAA AGTAGGCAAG
GTTAACCAA CGTTGAATTT TTAGACAAAC GTACTGAGAA AAGTCACTT TCATCCGTTG

1981 AGAAATTAA ATTCAGAAAT ATCTCACCTA ATGTCAGAGG TAATATTGAT AATTGTGTT
TCTTTAATTT TAAGTCTTTA TAGAGTGGAT TACAGTCTCC ATTATACTA TTAACACACAA

2041 TTACAAATAA TACATACAAC AATAATGAAA AATAAGTCCT ATCTATAGGC TCGTATCTCA
AATGTTTATT ATGTATGTTG TTATTACTTT TTATTCAGGA TAGATATCCG AGCATAGAGT

2101 TGCCCTATTTT TGGATGTATT TTCA
ACGGATAAAA ACCTACATAA AAAGT

FIGURE 47A

1	10	20	30	40	50	60
	1	2	3	4	5	6
	TGAAAATAC	ATCAAAAATA	GGCATGACAT	ACGAGCCTAT	AGATAGGACT	TATTTTTTAT
	ACTTTTATG	TAGTTTTTAT	CCGTACTCTA	TGCTCGGATA	TCTATCCTGA	ATAAAAATA
61	1	2	3	4	5	6
	TATTGTTOTA	TGTATTATTT	GTAAACACA	AATTATCAAT	ATTACCTCTG	ACATTAGGTG
	ATMACAACAT	ACATAATAAA	CATTTTGTGT	TTAATAGTTA	TAATGGAGAC	TGTAATCCAC
121	1	2	3	4	5	6
	AGATATTCTG	AATTTTAATT	TCTCTTGCC	ACTTTCAC	AAAAGAGTC	ATGCAACAG
	TCTATAAGAC	TTAAATTA	ACAGAACGGA	TGAAAGTGAC	TTTTTCTCAG	TACGTTTGTG
181	1	2	3	4	5	6
	ATTTTAAAGT	TGCAACCA	TTGCAAAATA	TTTTTTATC	CAACTTCAAT	GATAGGTATT
	TAAAAATTCA	ACGTTTGTT	AACGTTTAT	AAAAAATAG	GTTGAAGTTA	CTATCCATAA
241	1	2	3	4	5	6
	GCTGTTAATT	CTAAGATATG	CATTAAATGT	TTCAACTAAT	GGGTGTCAAA	CGAGATGTT
	CGACAATTAA	GATTCTATAC	GTAATTAA	AAGTTGATTA	CCCACAGTTT	GCTCTACAAG
301	1	2	3	4	5	6
	TGAAAATGAA	GGCAAAAGG	AGATCCACCT	TCTACTTTCA	TAAAGTTTCT	ATCTTCCTCT
	ACTTTTACTT	CCGTTTTTCC	TCTAGGTGGA	AGATGAAAGT	ATTTCAAA	TAGAAAGGAGA
361	1	2	3	4	5	6
	GCTGACTCAA	ATAAGCATTT	AATACATTTT	ATAACGAATT	AATTATGAAT	ATATTTCAAA
	CGACTGAGTT	TATTCGTAAA	TTATGTAAAA	TATTGCTTAA	TTAATACTTA	TATAAAGTTT
421	1	2	3	4	5	6
	TAAATAAATT	ATTCCCAAGT	GTTGAAGGAA	ATTCAGACTT	CTAATTTGCT	CTGATTCTGA
	ATTATTATA	TAAAGGTTC	CAACTTCCTT	TAACTCTGAA	GATTAAACGA	GACTAAGACT

FIGURE 47B

481 AACTAAACA AATGCTCTGT GAGAGTTTGC GTTCCAGTG AAGTAGCGTG AGAAATCCAA
TTGATTTTGT TTACGAGACA CTCTCAAACG CAAAGGTCAC TTCATCGCAC TCTTTAGGTT

541 GTCAGACAGC TACATGAAC TACATTTACC AGCTCTCTGC CAGACACCCAG TGCACGATAG
CAGTCTGTGG ATGTACTTTG ATGTAATGG TCGAGAGACG GTCTGTGGTC ACGTGTATC

601 CGCAGAACAT GTAGCTAGAT CTCAGTCATA GCTNNNNNNN NNNNNNNNNN AGACCTTGCA
GCGTCTTGTA CATCGATCTA GAGTCAGTAT CGANNNNNNN NNNNNNNNNN TCTGGAAACGT

661 GTTGGCTTTT AACCTGAAGG AGATAAGGCA AGATTCCAGG GTTTATTAG AGAAATTACA
CAACCGAAA TTGGACTTCC TCTATTCCGT TCTAAGGTCC CAAATAAATC TCTTTAATGT

721 GGATCTGGGA ATAAAGTAGT TACAAAATTA GTCCCAACC AGCTTTCATG GAGCTTTCAA
CCTAGACCCCT TATTTTCATCA ATGTTTTAAT CAGGGGTTGG TCGAAAGTAC CTCGAAAGTT

FIGURE 47C

781 TTATTAAATTA TTCTAGTTCT TAATCGCATG CATACAATGC ACATACATAT ATACATGCGAT
 AATAATTAAAT AAGATCAAGA ATTAGCGTAC GTATGTTACG TGTATGTATA TATGTACGTA

841 ATTAAATAC ATGATGGAC GCAACCGAA ATAAGATTCC ACCTGTGCGAT AAAACAGAAA
 TAATTTTATG TACTAACCTG CGTTTGCCTT TATTCTAAGG TGGACACGTA TTTTGTCTTT

901 GACTTGGTTA GAGTGAGGGA TCAGGAACA CCACACTGAG GACGAGATGN NNNNNNNNN
 CTGAACCAAT CTCACTCCCT AGTCCTTTGT GGTGTGACTC CTGCTCTACN NNNNNNNNN

961 NTAGTGGTG GGGGGCGGAC ATCAATAAAG AACTCTTCTG TGTCAGCCAC TGAGCACGGA
 NATCACCCAC CCCCCGCGCTG TAGTTATTTT TTGAGAAGAC ACAGTCGGTG ACTCGTGCCCT

1021 ATAAAGGGAT GAGAGTGAGG GCAANTACCA GAAGAATAAA ATCCTTTTAA GAGATGAAGA
 TATTTCCTTA CTCTCACTCC CGTTNATGGT CTCTCTTATT TAGGAAAATT CTCTACTTCT

1081 TTGTTATGAG CACAGTGTGT GNTTCAAAA ATCTTTTAAAC AACCCCAAGG TOAAGCTAAT
 AACAACTACTC GTGTCAACACA CCNAACTTTT TAGAAAATTG TTGGGGTTCC ACTTCGATCA

1141 TGAAGATAT TTGAATTGTG TTAACCCAT CTGGTCCTAG CCCTATTCTT TGAATCCCGA
 ACCTTCTATA AACTTAAACA AATTGGGTA GACCAGGATC GGGATAAGAA ACITAGGGCT

FIGURE 47D

1201 AAGAGGGTCA AGAATTCCGA GCAGGAGTGG ACTACCTGGT GATACCTTAG ACTAGTCCTG
TTCTCCCCAGT TCTTAAGGCT CGTCCTCACC TGNTGGACCA CTATGGAATC TGATCAGGAC

1261 TGTATTAAAG TCCAATGAGG AGTATCTTGG TAAATAATAA AATAAAGTCC CGAAATCCTC
ACATAAATTTC AAGTTACTCC TCATAGAACC ATTTTATTTAT TTATTTTCAGG GCTTTTAGGG

1321 AGTACTGTGC TAGGAGATTI ACATGCTATA TTTTACTA TNNNNNNNNT AATTGTCAGA
TCATGACACG ATCCTCTAAA TGTACGATAT AATAAATGAT AHHNNNNNNNA TTAAACGTCT

1381 TAATATTATC CTCATCATAA AATAGGGTAA CTACGCTGA GAGGGACTCG GTAACCTGTT
ATTATAATAG GAGTAGTATT TTATCCCATTT GATTGCGACT CTCCTGAGC CATGGAACAA

1441 CAAGGCCACT AAGAAGTGGC AAAGTCAAAA CTGGAATTTT AATAAAGAG TCTAGCTTGC
GTTCCGGTGA TTCCTCACCG TTTCAGTTT GACCTTAAA TTATTTTCTC AGATCGAAGC

1501 CTGTGTGGTT CTGCTTTTCT TAGAAAGTTG GANNAAGTCT CANATCAGTA CCCAGGAAA
GACACACCAA GACGAAAAGA ATCTTICAAC CTNNTTCAGA GTNTAGTCAT GGGTCCTTTT

1561 ACAGCAAAAG ACCCGCTGGT AAAGACCCTGT CCAGATTGCT GACCTGGTTC ACACANHTCC

FIGURE 47E

TGTCGTTTTC TGGGCGACCA TTTCTGGACA GGTCTAACGA CTGGACCAAG TGTGTHNAGG

1621 AAGCTTGCCT CTGTTACTTC CAAGGAAGAA AGAATGCACA GAGAGGTAAJ AAAACAACA
TTCGAACGGA GACAATGAAG GTTCCTTCIT TCTTACGTGT CTCGCCATT TTTTGTTTGT

1681 AACCAAAACA AACAAACA AACAAACA AACAAACA AAGCAAAA AAACTTCCTC
TTGGTTTGTT TTGTTTGT TTGTTTGT TTGTTTGT TTCGTTTTT TTTGAAGGAG

1741 TGTCTTGCG GGTCCAGCA CTTGGAACCT TCCTACGTCC TANTTTCAGG TTCCTCAGT
ACAGAACGTC CCGAGGTCGT GAACCTTGA AGGATGCCAG ATNAAGTCC AAGACAGTCA

1801 TCTACCCCTCA ACCTGAGTGA CTGTCCTACC AGCAGCTTGT CGAGAACTCA GCCCTGCACC
AGATGGGAGT TGGACTCACT GACAGGATGG TCGTCGAACA GCTCTTGAST CCGGACCTCG

1861 GTTCCCAGCT ACCCTCCTCC TAACTCGAGG GGTGCT
CAAGGGTCGA TGGGAGGAGG ATTGAGCTCC CCACGA

FIGURE 48A

1	10	20	30	40	50	60
	OGATICTGTT	GAGCCCTAGC	TCATTATGAT	GTCCTGTTGT	CCTACCCCAA	TAAGACTCAT
	CCTAAGACAA	CTCGGGATCG	AGTAATACTA	CAGGACAACA	GOATGGGTTT	ATTCTGAGTA
61	CCCAACTACA	TCFCAATAAT	TAATGAAGAT	GGAAATGAGG	TAAATAATTA	ATAATAAAT
	GGGTGATGT	AGAGTTATTA	ATTACTTCTA	CCTTTACTCC	ATTTTATTAT	TATTTATTA
121	AAAGAAACA	TTCCCCCCCC	TTTATTATTT	TTTCAATAC	CTTCTATGAA	ATAATGTTCT
	TTTTCTTTGT	AAGGGGGGGT	AAATAATAAA	AAAGTTTATG	GAAGATACTT	TATTACAAGA
181	ATCCCTCTCT	AAATATTAAAT	AGAAATCAAT	ATTATTGGAA	CTGTGAATAC	CTTTAATATC
	TAAGGAGAGA	TTTATAATTA	TCTTTAGTTA	TAATAACCTT	GACACTTATG	GAAATTATAG
241	TCATTATCCG	GTGTCAACTA	CTTTCCTATG	ATGTTGAGTT	ACTGGGTTTA	GAAGTCGGGA
	AGTAATAGGC	CACAGTTGAT	GAAAGCATAC	TACAACTCAA	TGACCCCAAT	CTTCAGCCCT
301	AATAATGCTG	TAAANNNNNN	AGTTAGTCTA	CACACCAATA	TCAAAATATGA	TATACTTGTA
	TTATTACGAC	ATTNNNNNN	TCAATCAGAT	GTGTGGTTAT	AGTTTATACT	ATATGAACAT
361	AACCTCCAAG	CATAAAAGAA	GATACTTTAT	AAAAGAGGTT	CTTTTTTTCT	TTTTTTTTTT
	TTGGAGGCTC	GTATTTTCT	CTATGAAATA	TTTTCTCCAA	GAAATAAGA	AAAAAANA

FIGURE 48B

421 TCCAGATGGA GTTCACTCC TGTCAGGCAQ QCNQAGTGCA GTGTGCCAT CTCQGCAC
AAGTCTACCT CAAAGTGAGG ACAGTCCGTC CQCTCAGGT CACCACGGTA GAGCCQAGTG

481 TQCAACCTCC ACCTCCCATG TTCAAGGQAT TCTCCTTCT CAGTCTCCTG AQTAGCTQGG
ACGTTQGAQ TQGAQGTAC AAGTTCCCTA AGAGGAAGGA QTCAGAGQAC TCATCGACCC

541 ATTACAGGTG TGCACCACCA CACCAGCTA ATTTTGAT TTTAANTAGA QACAGGGTTT
TAATGTCCAC ACQTGQTQGT QTQGTGAT TAAACATA AAATTATCT CTGTCCCAA

601 CATCGATGTT GGCCAGGCTA GTCTCQACT CCTGACCTCT AGGTGATCCA CCCQCTCAG
GTAGCTACAA CCGTCCQAT CAGAQTQA GQACTGGAGA TCCACTAGGT QGQCGQAGTC

661 CCTCCCAAAG TTGTAGAATT ACACGTGTGA QGCACTGCTC TGQCCAGGAG ATACATTTT
GGAGGGTTTC AACATCTTA TGTGCACACT CCGTGACGAG ACCQCTCCTC TATGTAAAAA

721 GATAGGTTTA ATTTATAAAG ACACGTGCACA GATTGGACT TGCTGGGAAA TCACGATCCA
CTATCCAAAT TAAATATTTC TGTQACGTGT CTAACCTCA ACGACCTTT AQTGCTAQT

FIGURE 48C

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781 GTATGCATTT GACCCAGCAA TTTTATTGG TACTAATGA TTATAICTCA ATTGATCAGG
CATACGTAAA CTGGGTCGT AAAAATAACC ATGAATTACT AATATAGAGT TAACTAGTCC

841 TTGAACTCTG TGCAGAGAA TTGTGTGTG ACATTTBAGA GGACAGTTTG GAGGCAAGGT
AACTTGAGAC ACGCTTCTA AACACACACC TGTAACCTCT CCGTCAAAAC CTCCGTTCCA

901 ATTTTAGTAG ATTAAAGAA TTTGAATCTT GTTGCAAGT TGGGCGATAT ACTGAGAAAG
TAAATCAIC TAAATTCTT AAACCTTAAA CAAACGTTCA ACCCGGATTA TGACTCTTTC

961 AGAAGACAAT GCAGATAAT TGTATATTT TGTATGATGT ATGTTCAATA TGAAAGATCA
TCTTCTGTTA CGTCTATTTA ACTATATATA TAATACTACA TACAAGTTAT ACTTCTAGT

1021 CAAATATAA CATACATNNA TCTTACTTAA CATACCTCAG TTTTAGAGCT ACCGTATGTA
GTTTATATTT GTATGTANNT AGAATGAAAT GTATGAGAGC AAAATCTGA TGGCATAACAT

1081 GAAGAGTCCA TTTCTATTTA GGTAGTTCC TTTAGTCCTT TTATTACTGG GCACCTCTAA
CTTCTCAGGT AAAGATAAAT CCATTCAGG AAATCAGGAA AATAATGACC COTGAGAAAT

1141 TTACATGTAG CTTGAATAT GTCCAGTTTG AGCAGTGMAC TGAATATGTC ATGTGATTAA
AATGTACATC GAACCTTTATA CAGTCAACAC TCGTCACTTG ACTTTTACAG TACACTAATT

1201 GTACATATAT AATTTTTTT CATAGTAGGT CAATAACCTC CTTTATTGAA CTAAATGAATC
CATGTATATA TTAAATAAAA GATCATCCA GTTATTGGAG GAAATATAGT GATTACTTAG

1261 AGTTCTCTAA TGATTATACG
TCAAGAGATT ACTAATATGC

FIGURE 49A

1	10	20	30	40	50	60
	1	2	3	4	5	6
	AATCAAAATA	AAACAGTTAA	AGTTTGATTA	CTATAATCAA	ACACAAAAAA	AATGAATATT
	TTAGTTTAT	TTTGTCATT	TCAAACTAAT	GATATTAGTT	TGTGTTTTTT	TTACTTATAA
61	10	20	30	40	50	60
	1	2	3	4	5	6
	ATCTTTTATG	TCAGTAGAGG	GTGAATGAAT	CCTTCAGGAT	TTTGATGATA	GTATCAGATA
	TAGAAAAATAC	AGTCATCTCC	CACTTACTTA	GGAAGTCCTA	AACTACTAT	CATAGTCTAT
121	10	20	30	40	50	60
	1	2	3	4	5	6
	CCCAGCACTA	TGCTAGAAGT	TGTGAAGAAT	TCACGAGATG	AATAAATCAC	AGATTCTGTC
	GGGTCGTGAT	ACGATCTTCA	ACACTTCTTA	AGTGCTCTAC	TTATTTAGTG	TCTAAGACAG
181	10	20	30	40	50	60
	1	2	3	4	5	6
	CTCAAAATGG	TTAGATCTAT	TCAGGAAACA	AAGCTAAAAA	AACCCACCA	ATAACTAAAA
	GAGTTTTACC	AATCTAGATA	AGTCCTTTGT	TTGATTTTTT	TTGGGGTGGT	TATTGATTTT
241	10	20	30	40	50	60
	1	2	3	4	5	6
	ATCAACCANA	TGAAAAACA	CAATCATAAA	ATAAGTAAGT	ACCTATAGAA	AGAAAAGCTC
	TAGTTGGTTT	ACTTTTGTG	GTTAGTATTT	TATTCATICA	TGGATATCTT	TCTTTTCGAG
301	10	20	30	40	50	60
	1	2	3	4	5	6
	AGAGGAGGTA	AAAAGATAAC	TCTTCCAAAA	GGAATACTAT	ATACTGTAAA	CTGTGTACTG
	TCTCCTCCAT	TTTTCTATIG	AGAAAGGTTTT	CCTTATGATA	TATGACATTT	GACACATGAC
361	10	20	30	40	50	60
	1	2	3	4	5	6
	ATAGAAGGAA	GAATTAGAAA	NNNNNNNNTG	TAAGTGGCAT	ACATACTAAG	CTAOTGTGAA
	TATCTTCCTT	CTTAATCTTT	NNNNNNNNAC	ATCACCCTTA	TGTATGATTC	GATCACACTT

FIGURE 49B

421 CACAAGCCTA AATATGTAGT TGCTTCACAG AAGGTTAGAA GTAAATTAAC CTCATGAATT
GTGTTCCGGAT TTATACATCA ACGAAGTGTC TTCCAATCTT CATTTAATIG GAGTACTTAA

481 TCTTGAGAGA ACTTGTAAGG ACTAAGCTTT CGATTITGGA GAAAGATTTT AATACCAANT
AGAACTCTCT TGAACATTCC TGATTCGAAA GCIAAAACCT CTTTCTIAAAA TTATGGTTTA

541 AAAAAGTACC TTTGTTTGGT AATCTCAATC ATTATAATAG TGCTTAGATA ATACCTAGGA
TTTTTTCATGG AAACAAACCA TTAGAGTTAG TAAATATTATC ACGAATCTAT TATGGATCCT

601 ACAAAATTAA TATTAAATTT ACTTTAAAAA AAAGTACATG ATTGGGGAAT CACAACCTGGC
TGTTTAATTT ATAATTAA TGAAATTTTT TTTCATGTAC TAACCCCTTA GTGTTGACCG

661 CTTACTAGAT TCTCTNNNNN NATATGCACT GAAAGAATG AAAAACACTG AACCAATAT
GAATGATCTA AGAGANNNNN NTATACGTGA CTTTCTTAC TTTTGTGAC TTGGTTTATA

721 NTGTTTTTTT AAGTTTAAA TTAATTGGA AAAAATAGT AAGGAATATC AGAAGCAAAA
NACAAAAAAA TTCAAATTTT AATTNACCT TTTTATTATCA TTCCTTATAG TCTTCGTTTT

FIGURE 49C

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781 AAATAAAATG AAAGCAAGAA TCCTCAGAGG TAGCAGCAA TTTGGCTTTG CTTAGATGGA
   TTTATTTTAC TTTCTGTTCTT AGGAGTCTCC ATCGTGCTTT AAACCGAAAC GAATCTACCT

841 TCTATCAAAG CTAIGGCCCA TGAAGAAGAT TCAGGAGTTA GTTTAAAGCT GGTTCACATA
   AGATAGTTTC GATACCGGGT ACTTTTCCTA AGTCCTCAAT CAAATTTTGA CCAAGTGTAT

901 ATGGAATCTA GCAGAAGACT GTGCATAAAG GTGGTCTAAG AACAAACAATA TCCTGACCAAG
   TACCTTAGAT CGTCTTCTGA CACGTATTTT CACCAGATTTC TTGTTGTTAT AGGACTGGTC

961 GTGAGGGGGC TCACNCTNAA TNCCAGCACT TTGGGAGCCC AAGGTGGGTG GATCACGAGG
   CACTCCCCCG AGTGNGANTT ANGGTCGTGA AACCCTCGGG TTCCACCCAC CTAGTGCTCC

1021 TCAGGAGTTT GAGACCAGCC TGACCAACAT GGTGAAACCG CGTCTCTACT AAAAATAGAA
   AGTCCCTCAA CTCTGGTCCG ACTGGTTGTA CCACTTTGGC GCAAGAGATGA TTTTATCTTT

1081 AATTAGCCG NGCCTACGTG CTTCTAATCC CAGCTGAAC T CAGGAGACTG AGACAGGAGA
   TTTAATCGGC NCGGATGCAC GAAGATTAGG GTCGACTTGA GTCCCTCTGAC TCTGTCCTCT

1141 ATCACTTGAA CCCAGCATGC AAGCTTNNNN NNGCCACTGC ACTCCAGCCT AGGGTGCAAA
   TAGTGAACCT GGGTCGTACG TTCGAANNNN NNCGGTGACG TGAGGTGCGA TCCCACGTTT

1201 AAAAAAAAA ANGACACATT ACTCAGGTAA GGTAAATCAAT AA
   TTTTTTTTTT TNCGTGTGTA TGAGTCCATT CCATTAGITA TT

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FIGURE 50A

[illegible]

FIGURE 50B

|||||
- ATTTAAATAATCCCTTTCGACTGTAGAACAAATAGGAATTTGGCCTGT -
|||||
- GGGGTCTACTTGCCTTATTATATTGTAAAGCTAGTGGTAGGAATAGCAAA -
|||||
- GGGGTCTACTTGCCTTATTATATTGTAAAGCTAGTGGTAGGAATAGCAAA -
|||||
- TGCTCACTACCACTAATAAGAACATTTTCTAAATCTGATGTCTGAGGATT -
|||||
- TGCTCACTACCACTAATAAGAACATTTTCTAAATCTGATGTCTGAGGATT -
|||||
- TTTAGAGCTTATAGTAGCAAAAGAAAGGGAAATTTCTATCCGAGATGTC -
|||||
- TTTAGAGCTTATAGTAGCAAAAGAAAGGGAAATTTCTATCCGAGATGTC -
|||||
- CTTTGTGTAGGCCCTAATGAGAAAGGTTGAAGATAAAGTTCTGGTACTC -
|||||
- CTTTGTGTAGGCCCTAATGAGAAAGGTTGAAGATAAAGTTCTGGTACTC -
|||||
- ATTTAAGTGTAATAATTGAAATTTGATATTACCGAATCTGGAAACAACCAAT -
|||||
- ATTTAAGTGTAATAATTGAAATTTGATATTACCGAATCTGGAAACAACCAAT -
|||||
- TTAAATTAAGGAAGAAGACACTGTGTTTCT -
|||||
- TTAAATTAAGGAAGAAGACACTGTGTTTCT -

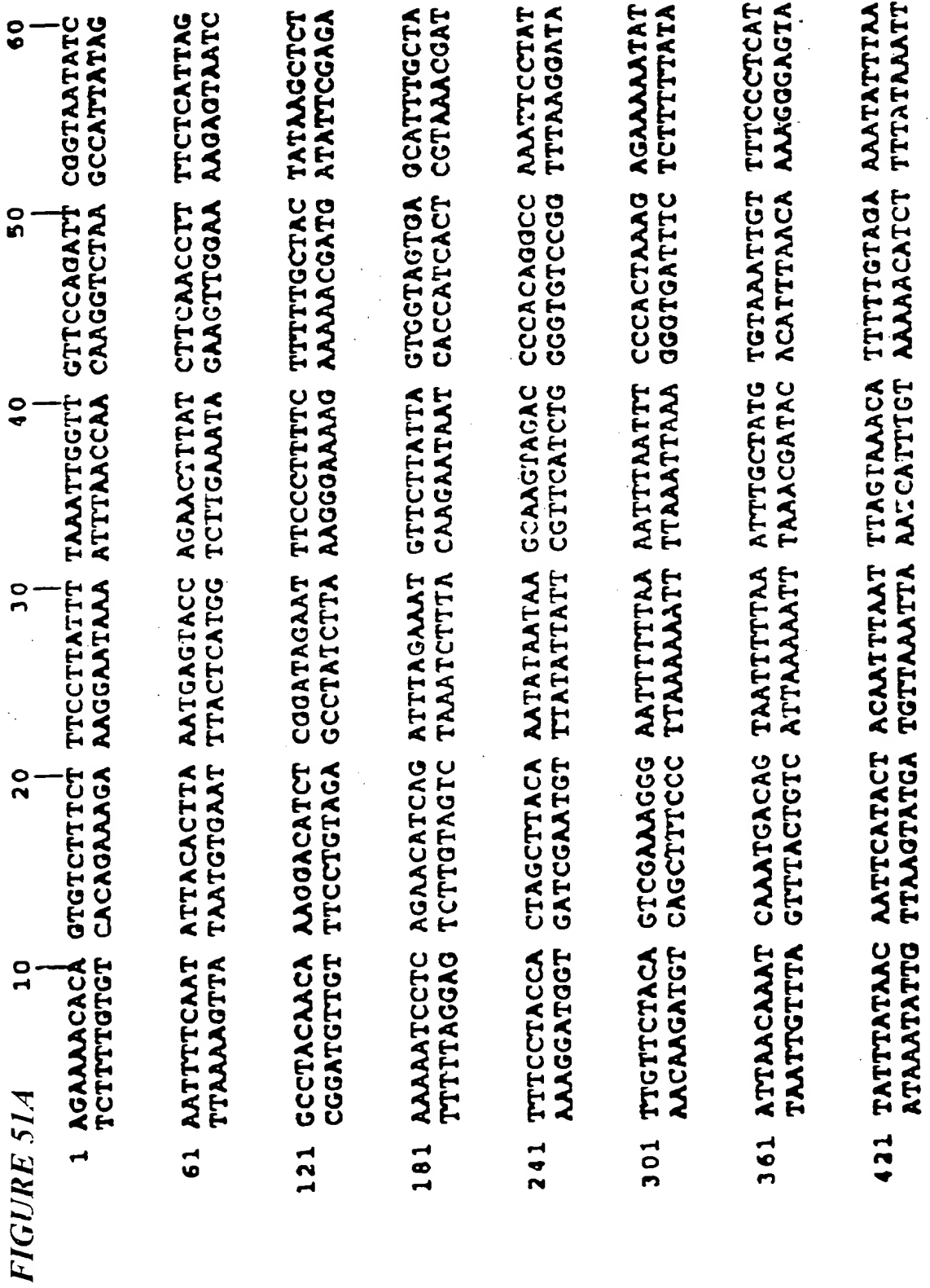


FIGURE 51B

481 AACAAAGATA CTGAAAGTTA ATATNAAACC CAGTGCAATG TTCTTGTAGG CCACAGCCAT
TTGTTTCTAT GACTTTCAT TATANTTTGG GTCACGTACG AAGAACATCC GGTCGCGTA

541 AACCTGTAAG CACAGAAATA TTGTCTCTGT TACTCTAAAC ATCTACACIG GCCAAATTCC
TTGGACATTC GTGTCTTTT AACCAAGACA ATGAGATTG TAGATGTGAC CGGTTTAAGG

601 AATGCTCGAA TTTAACCCCG GGATATAACC TAGTAATGT GTCTCTCTG TAAGGTGGG
TTACGAGCTT AAATTGGGG CCTATATTGG ATCATTTACA CAGGAGAGAC ATCCACCCG

661 ATGTCACAGA ATACAAGAA ATAATGGTAT TCATAAAGTT TTAAGAAAT GATTCTACAC
TACAGTGTCT TAGTTCTTT TATTACCATA AGTATTTCAA AATTCTTTA CTAAGATGTG

721 ATGTAAACC CACTATAACT TTTTACATTG GGGGAGAGAA AAAAGAGAT AATTTTACC
TACATTTGG GTGATATTGA AAAATGIAAC CCCCTCTCT TTTTCTCTA TTAATAATG

781 TT
AA

FIGURE 52A

	10	20	30	40	50	60
1	GATGCTATTT	GGCAATTTC	TTATTGACAG	TTTTGAAATG	TTAGGCTTTT	ATCTCCATTT
	CTACGATAAA	CCCGTTAAAG	AATAACTGTC	AAACTTTAC	AATCCGAAAA	TAGAGGTAAA
61	TTTAGTACTT	AAATTTTCCA	ACATGGGTGT	TGCTTGTTAT	TTTATCAGTA	TAAATAGAA
	AAATCATGAA	TTTAAAGGT	TGTACCCACA	ACGAACAATA	AAATAGTCAT	ATTTTATCTT
121	GAGTGGTTCT	GTTCTGGAAT	TTAGTATATA	CATGAGTATC	TAGTGTATGT	CAGCCATGAA
	CTCACCAAGA	CAAGACCTTA	AATCATATAT	GTACTCATAG	ATCACATACA	GTCGGTACTT
181	AATGAACCTT	TCAGATGTTT	AACCTCAGGG	AACCTAATTG	AGTCATTGCT	CCAGACATTG
	TTACTTGGAA	AGTCTACAAA	TTGAGTCCCT	TTGGATTAAAC	TCAGTAAACGA	GGTCTGTAAAC
241	TTGCTTTGAA	CCCACTATAT	TNNNNNNNCT	CGGGCAATGA	CTCAGTGTGG	CAAGGATACT
	AACGAAACTT	GGGTGATATA	ANNNNNNNGA	GCCCGTTACT	GAGTCACACC	GTTCCCTATGA
301	ACTGCAGGCC	TGTTTCTGGA	AGGCAC TGGA	CTCCTCTGAT	GCAAACTTTG	CCCAGGGACT
	TGACGTCCGG	ACAAGACCTT	TCCGTGACCT	GAGGAGACTA	CGTTTGAAAC	CGTCCCTGA
361	CCTTGATAGC	TCTTAAATAG	ATGCTGCACC	AACACTCTCT	TTCTTTTCTC	TCTTTTCTTT
	GGAACTATCG	AGAAATTATC	TACGACGIGG	TTGTGAGAGA	AAGAAAGAG	AGAAAAAGAA

FIGURE 52B

421 TATTCAATAT TAGACTACAA GCAGTCTAAG GACTTCTCAG GGTTTCTAGC TCTCTCTCAT
ATAAGTTATA ATCTGATGTT CGTCAGATTG CTGAAGAGTC CCAAGATCG AGAGAGAGTA

481 TTCACACAIG CTTTCTTAGT AAICTCTACT CAIATAICTT ACTOCTACGC TGGGGCCAGA
AAGTGTGTAC GAAAGGATCA TTAGAGATGA GIATATAGAA TGACGATGCG ACCCCGGTCT

541 TAACNNNNNN CTTCCATTTT GTTTTATCT CTATTCITCT TCCCCTCTG CTTTCATTAT
ATTGNNNNNN GAAGGTAAA CAAAATAGA GATAAGAAGA AGGGGAAGAC GAAAGTAATA

601 TGAACCTTTC TGCTTTCATT ATTGAACCTT TCCCAGATTG GTTCTGCTTA ACCTGGCATT
ACTTTGAAAG ACGNAAGTAA TAACTTTGAA AGGGTCTAAA CAAGACGAAT TGGACCGTAA

661 GGAACGTGTT CCTCTTCCCT GTGCTGCTTT CTCCCATTGC CATGTCCCTT TTTTITTTTT
CCTTGACAAA GGAGAAGGA CACGACGAAA GAGGGTAACG GTACAGGAAA AAAAAA

721 TTTTITTTTT TGAGACAGTG TCACTCTGTT GCCCAGGCTG GAGTGCAATG GTGCAATCTT
AAAAA

FIGURE 52C

781 GQCCACTGCA ACCCCGCGCT CCGGGTTCA AGTGATTCTC CTGCCTCAGC CTCCTGAGTA
CCGGTGACGT TGGGGGCGGA GGCCCAAGT TCACTAAGAG GACGGAGTCG GAGGACTCAT

841 GCTGGGATTA CAGGTGCCCA CCACTATGCC CGGCTGATTT TTGTATTTT AGTAGAGATN
CGACCCCTAAT GTCCACGGGT GGTGATACGG GCCGACTAAA AACATAAAA TCATCTCTAN

901 NNNNNNNNTT CACCATNGCT GATCAGGCTG GTCTCGAACT CCTGACCGCA GTGANTCCGC
NNNNNNNAAA GTGGTANCGA CTAGTCCGAC CAGAGCTTGA GGA CTGCGGT CACTNAGGCG

961 CCTCCTTGCC CTCCCAAGT GCTGAGATTA CAGGCATGAG TCACTGCGNC CAGCCACCAT
GGAGGAACCG GAGGGTTTCA CGACTCTAAT GTCCGTACTC AGTGACCGNG GTCCGTGGTA

1021 TATTCTCTAG AGGTGAGAGA ACACTGGCTC TTCTAACAAAG TTGAAATTTG ATAGAGACC
ATAAGAGATC TCCACTCTCT TGTGACCGAG AAGATTGTTT AACTTTAAAC TATCTCTGO

FIGURE 53A

	10	20	30	40	50	60
1	CACAAATAA GTGTTTIT	GATTATTAGC CTAATAATCG	CACAAATAA GTGTTTIT	CCTTGAAGTA GGAACATCAT	ACGCATTAA TCCGTAATTT	ATGTTAATGG TACAATTACC
61	ATTCACCTTA TAAGTGAAAT	TTGAOCATCT AACTCGTAGA	GCTCATAATA CGAGTATTAT	CTTTAATGAG GAAATTACTC	TGCAAGTGCC ACGTTTCACG	TTTGAATATA AAACTTATAT
121	ATACGTCATT TATGCAGTAA	TAAACCTTAC ATTGGAATG	CATAATTCIG GTATTAAGAC	AGGAATIGCT TCCTTAACGA	ACCTCCACTT TGGAGGTGAA	CACAGATGGG GTGTCACCC
181	GCACAGGAGG CGTCTCCTCC	CTTAGATAAC GAATCTATTG	ATGCCCAAG TACGGGTTTC	TCATGCTTCT AGTACGAAGA	AGTAAATGGA TCATTTACCT	TATAATTAAAG ATATTAAATTC
241	ATTCAAAATTA TAAGTTTAAT	TTGATAAGAA AACTATTCTT	TTTGATCTGC AACTAGACG	CTTACCAGTA GAAATGGTCT	TCTAGTAGTA AGATCATCAT	AATCTAAAG TTAGATTTTC
301	CGCTTTCCAG GGGAAAGGTC	AGCATGTGCT TCGTACACGA	GTGATAGAG CAACTATCTC	CTTATGCTCT GAACTACAGA	AATCTCTGGA TTGAGAGACT	AATTTTCCAT TTAAAGGTA
361	TCTTATTGTT AGAATAAACA	CTCACTGGTA GAGTGACCAT	TATAGTTATT ATATCAATAA	TTTTACTACT AAATGATGA	TTCATACACC AGTATGTGG	TACTAAGAAAG ATGATTTCTTC

FIGURE 53B

421 ACAGGAGGAT CAAAGATAGG ATTCATTTA GAATGCCCTAA AGCTTCACGT ATTTAATTC
TGTCCTCCTA GTTCTATCC TAAAGTAAAT CTTACGGATT TCGAAGTGCA TAAATTTAAG

481 AGAATAAGAT TCAGGCAGAC CACCAGTATA TGCCATGCTC CCTGGTTATC TTTCAGCAGG
TCTTATTCTA AGTCCGCTCG GTGGTCATAT ACGGTACCAG GACCCAATAG AAAGTCGTCC

541 TGACCGAGAA AGAAACATG GTAATGTITA TGAATCGTG GGTTCCTGTA GTTCACTTC
ACTGGCTCTT TCTTTGTAC CATTACAAAT ACTTIACCAC CCAAGAACAT CAAAGTGAAG

601 AACATATCTG CCTTTACIGT ATTAAGATGA TGGATTAACT TATTCCTGAT ATGGGCATGT
TTGTATAGAC GGAATGACA TAATICTACT ACCTAATTGA ATAAGAACTA TACCCGTACA

661 AAAACAATAT ACTTTTACTA AACAGCTACA GAGAGACAA TGTGTTTCCA GACAACTTA
TTTTGTATATA TGAATAATGAT TTGTCGATGT CTCCTGTTT ACACAAAGGT CTGTTGAAT

721 AGAGACTGAG TGTTCAAACT GAATAATCTC GACCTTAATT GAACTATAT TTTATGAAAT
TCTCTGACTC ACAAGTTTGA CTTATTAGAG CTGGAATTAA CATGATATA AAATACTTTA

FIGURE 53C

781 CCAGCTGTAA GGCAAAACA GACTTCTTTG GGCTACCAC GGCATTTTG TTCCTGTTAN
 GTCGACATT CCGTTTGT CTGAAGAAAC CCGATGGTG CCGTAAAC AAGGACAAATN

 841 NNNTACTCCA AACCTTAAAC CCACGTCCAC TTAAATTAAG GCTGGAAT AAATGTCATT
 NNNATGAGGT TTGGAATTG GGTGCAGGTG AATTATTAC CGACCTTTA TTTACAGTAA

 901 ATCTGATATT ATACTGAGAT GTTAGTTAT GAAATCAAA GTGGAGAAT TCAATCTGTC
 TAGACTATAA TATGACTCTA CAAACATA CTTTAGTTT CACCTCTTA AGTTAGACAG

 961 CTGTAAGCTT TCTCTGCGGT CACGACCTC ATGCACTCAG GCTGTGCGT GCAGCATGCT
 GACATTGAA AGAGACGCCA GTGCTGGAG TACGTGAGTC CGACACGCCA CGTCGTACCA

 1021 CTGTCAATGC TGTCTTCTC TGCCTGTACA CGGGTGGTG TTCCTGTCTA CCTGTTTGAG
 GACAGTACAG ACAAAAGAAG ACGACATGT GCCCACCAC AAGCACAGAT GGACAACTC

 1081 GAAATATGAA TACGTNNNNN NCTAGAATCT ACTGCACATG CAATAAGGA ACAATCAGTA
 CTTTATACTT ATGCANNNNN NGATCTTAGA TGACGTGTAC GTATTCCTT TGTTAGTCAT

 1141 AGAATCAGTT TCTCGTGGAA AATCATTAG AATTAACATC TCGTTTTAA ATGCTCTATC
 TCTTAGTAA ACAGCACCTT TTAAGTAA TC TTAATTGTAG AGCAAATTT TACGAGATAG

FIGURE 53D

1201 AAGTGTAA TAATCCCTCT CTCTTTCCC TTTTCACTA AGGAGTTTGT ATATTAACA
TTTCACATTT ATTAAGGAGA GAGAAAGGG AAAAGTGAT TCCTCAAACA TATAATTGT

1261 GAATTCAAG TAATGTATTA TAAATTTATT TAANNATTT ACAATAAAAT GCCACGTATA
CTTAAAGTTC ATTACATAAT ATTTAAATAA ATTNNATAA TGTATTTTA CGTGCAATAT

1321 AGCATCAAGC AACATGANN NNNCATTTGGT AGAAGCACA ATACATAGTC AAAACAGCAG
TCGTAGTTCC TTGTACTNNN NNGTAACCA TCTTTCGTGT TATGTATCAG TTTTGTGTC

1381 AGTATTAAAT AACAGAAAT TITGCAAAAG GCAAGTAAAG AATATACATA TACTTAATTA
TCATAAATTA TTTGTCTTTT AAACGTTTC CGTTCATTTT TTAATATGTAT ATGAATTAAAT

1441 TACATAAAAT ATTGATACAG GAGGTAGAA GAAATTTAGT AAGCAGATAA TGGGGGCAAC
ATGTATTTTA TAACTATGTC CTCCATCTTT CTTTAAATCA TTGCTCTATT ACCCCCGTTG

1501 AGAGTCCTCA GCAGAGCTTC CCTTCTAACA AAAAGCAGCC CAATAAATTA TTTTTTTTTT
TCTCAGGAGT CGTCTCGAAG GGAAGATTGT TTTTCGTGCG GTTATTTAAT AAAAATAAAA

1561 CTAACAAAA GCAGCCTGAA AATCGAGCT GCAACATAG ATCAGCAATC GCTGAAAGT

FIGURE 53E

GATTOTTTTT CGTCGGACTT TTAGCTCGA CGTTGTATC TAATCGTTAG CCGACTTTCA

 1621 GCGGAGAAAT GCTGOCAGCT GTGCCAATAG TAAAGGGCTA CCTGGAGCCO OGCGCGTGCC
 CGCCCTCTTA CGACCGTCGA CACGGTTATC ATTCCCGAT GGACCTCGGC CCGCGCACCG

 1681 TCACGCTGTA ATCCCAGCAC TTTGGGAGGG CGAGGCAACG CGGATCACCT GAGGTCGGGA
 AGTGGGACAT TAGGGTCGTG AAACCCICCC GCTCCGTTGC GCCTAGTGGG CTCCAGCCCT

 1741 GTTTGAGATC AGCCCGACCA ACATGGAGAA ACCCCGTCTC TACTAAAAA AAAAAAAA
 CAAACTCTAG TCGGGCTGGT TGTACCTCTT TGGGCGAGAG ATGATTTTTT TTTTTTTTTT

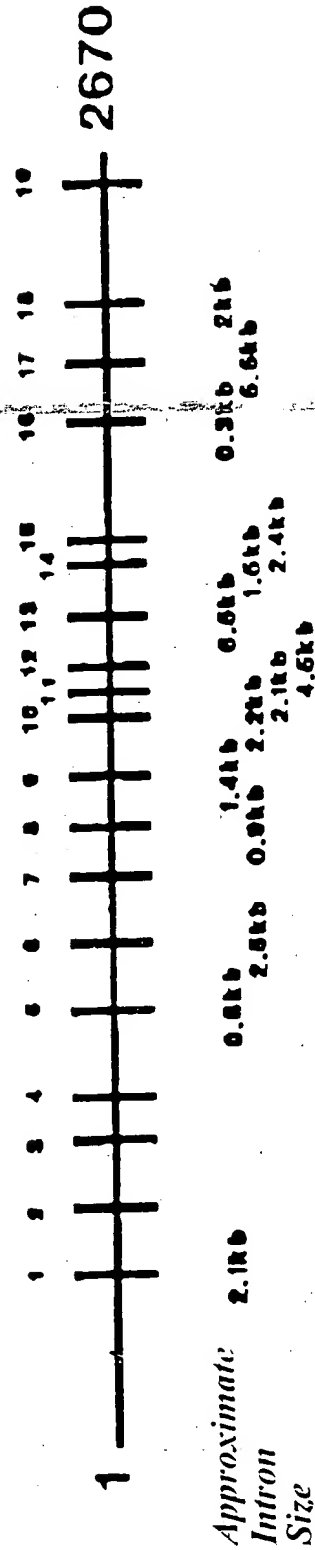
 1801 AAAGGCAAAA AATGAGCCGG GCATGGTGGC ACATGCCCTG CACATCCCAG CTGAGGCAGG
 TTTCCGTTTT TTA CTGCGCC CGTACCACCG TGTACGGGAC GTGTAGGCTC GACTCCGTCC

 1861 AGAATTCACT TGAACCTGGG AGGTAGAGAT TCGGGTGAAG CGAGATCACG TCATTGCACCT
 TCTTAAGTGA ACTTGGACCC TCCATCTCTA ACGCCACTTC GCTCTAGTGC AGTAACGTGA

 1921 CCAGCCTGGG CAAAAGAGGC AAACTTAGT CTCAAAAA AAANNCAA GAAAAA
 GGTCCGGACCC CTTTTCTCG TTTTGAATCA GAGTTTTT TTTTNGTTT CTTTTT

FIGURE 54

Genomic Organization of PSM Gene



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FIGURE 55A

10 20 30 40
* * * * *
CTC AAA AGG GGC CGG ATT TCC TTC TCC TGG AGG CAG ATG TTG CCT CTC

50 60 70 80 90
* * * * *
TCT CTC GCT CGG ATT GGT TCA GTG CAC TCT AGA AAC ACT GCT GTG GTG

100 110 120 130 140
* * * * *
GAG AAA CTG GAC CCC AGG GTG GTT TAT AAA ATC CTC CAA TGA AGC TAC

150 160 170 180 190
* * * * *
TAA CAT TAC TCC AAA GCA TAA TAT GAA AGC ATT TTT GGA TGA ATT GAA

Met Lys Ala Phe Leu Asp Glu Leu Lys>

200 210 220 230 240
* * * * *
AGC TGA GAA CAT CAA GAA GTT CTT ATA TAA TTT TAC ACA GAT ACC ACA

~~Ala Glu Asn Ile Lys Lys Phe Leu Tyr Asn Phe Thr Gln Ile Pro His>~~

FIGURE 55B

250 260 270 280
 * * * * *
 TTT AGC AGG AAC AGA ACA AAA CTT TCA GCT TGC AAA GCA AAT TCA ATC

Leu Ala Gly Thr Glu Gln Asn Phe Gln Leu Ala Lys Gln Ile Gln Ser>

290 300 310 320 330
 * * * * *
 CCA GTG GAA AGA ATT TGG CCT GGA TTC TGT TGA GCT AGC ACA TTA TGA

Gln Trp Lys Glu Phe Gly Leu Asp Ser Val Glu Leu Ala His Tyr Asp>

340 350 360 370 380
 * * * * *
 TGT CCT GTT GTC CTA CCC AAA TAA GAC TCA TCC CAA CTA CAT CTC AAT

Val Leu Leu Ser Tyr Pro Asn Lys Thr His Pro Asn Tyr Ile Ser Ile>

390 400 410 420 430
 * * * * *
 AAT TAA TGA AGA TGG AAA TGA GAT TTT CAA CAC ATC ATT ATT TGA ACC

Ile Asn Glu Asp Gly Asn Glu Ile Phe Asn Thr Ser Leu Phe Glu Pro>

440 450 460 470 480
 * * * * *
 ACC TCC TCC AGG ATA TGA AAA TGT TTC GGA TAT TGT ACC ACC TTT CAG

Pro Pro Pro Gly Tyr Glu Asn-Val Ser Asp Ile Val Pro Pro Phe Ser>

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FIGURE 55C

490 500 510 520
* * * * *
TGC TTT CTC TCC TCA AGG AAT GCC AGA GGG CGA TCT AGT GTA TGT TAA

Ala Phe Ser Pro Gln Gly Met Pro Glu Gly Asp Leu Val Tyr Val Asn>

530 540 550 560 570
* * * * *
CTA TGC ACG AAC TGA AGA CTT CTT TAA ATT GGA ACG GGA CAT GAA AAT

Tyr Ala Arg Thr Glu Asp Phe Phe Lys Leu Glu Arg Asp Met Lys Ile>

580 590 600 610 620
* * * * *
CAA TTG CTC TGG GAA AAT TGT AAT TGC CAG ATA TGG GAA AGT TT[†] CAG

Asn Cys Ser Gly Lys Ile Val Ile Ala Arg Tyr Gly Lys Val Phe Arg>

630 640 650 660 670
* * * * *
AGG AAA TAA GGT TAA AAA TGC CCA GCT GGC AGG GGC CAA AGG AGT CAT

Gly Asn Lys Val Lys Asn Ala Gln Leu Ala Gly Ala Lys Gly Val Ile>

680 690 700 710 720
* * * * *
TCT CTA CTC CGA CCC TGC TGA CTA CTT TGC TCC TGG GGT GAA GTC CTA

Leu Tyr Ser Asp Pro Ala Asp Tyr Phe Ala Pro Gly Val Lys Ser Tyr>

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FIGURE 55D

730 740 750 760
* * * * *
TCC AGA TGG TTG GAA TCT TCC TGG AGG TGG TGT CCA GCG TGG AAA TAT

Pro Asp Gly Trp Asn Leu Pro Gly Gly Gly Val Gln Arg Gly Asn Ile>

770 780 790 800 810
* * * * *
CCT AAA TCT GAA TGG TGC AGG AGA CCC TCT CAC ACC AGG TTA CCC AGC

Leu Asn Leu Asn Gly Ala Gly Asp Pro Leu Thr Pro Gly Tyr Pro Ala>

820 830 840 850 860
* * * * *
AAA TGA ATA TGC TTA TAG GCG TGG AAT TGC AGA GGC TGT TGG TCT TCC

Asn Glu Tyr Ala Tyr Arg Arg Gly Ile Ala Glu Ala Val Gly Leu Pro>

870 880 890 900 910
* * * * *
AAG TAT TCC TGT TCA TCC AAT TGG ATA CTA TGA TGC ACA GAA GCT CCT

Ser Ile Pro Val His Pro Ile Gly Tyr Tyr Asp Ala Gln Lys Leu Leu>

920 930 940 950 960
* * * * *
AGA AAA AAT GGG TGG CTC AGC ACC ACC AGA TAG CAG CTG GAG AGG AAG

Glu Lys Met Gly Gly Ser Ala Pro Pro Asp Ser Ser Trp Arg Gly Ser>

FIGURE 55E

970 980 990 1000
 * * * * *
 TCT CAA AGT GCC CTA CAA TGT TGG ACC TGG CTT TAC TGG AAA CTT TTC

Leu Lys Val Pro Tyr Asn Val Gly Pro Gly Phe Thr Gly Asn Phe Ser>

1010 1020 1030 1040 1050
 * * * * *
 TAC ACA AAA AGT CAA GAT GCA CAT CCA CTC TAC CAA TGA AGT GAC AAG

~~Thr Glu Lys Val Lys Met His Ile His~~ Ser Thr Asn Glu Val Thr Arg>

1060 1070 1080 1090 1100
 * * * * *
 AAT TTA CAA TGT GAT AGG TAC TCT CAG AGG AGC AGT GGA ACC AGA CAG

Ile Tyr Asn Val Ile Gly Thr Leu Arg Gly Ala Val Glu Pro Asp Arg>

1110 1120 1130 1140 1150
 * * * * *
 ATA TGT CAT TCT GGG AGG TCA CCG GGA CTC ATG GGT GTT TGG TGG TAT

Tyr Val Ile Leu Gly Gly His Arg Asp Ser Trp Val Phe Gly Gly Ile>

1160 1170 1180 1190 1200
 * * * * *
 TGA CCC TCA GAG TGG AGC AGC TGT TGT TCA TGA AAT TGT GAG GAG CTT

Asp Pro Gln Ser Gly Ala Ala Val Val His Glu Ile Val Arg Ser Phe>

1210 1220 1230 1240
 * * * * *
 TGG AAC ACT GAA AAA GGA AGG GTG GAG ACC TAG AAG AAC AAT TTT GTT

Gly Thr Leu Lys Lys Glu Gly Trp Arg Pro Arg Arg Thr Ile Leu Phe>

FIGURE 55F

1250 1260 1270 1280 1290
 * * * * * * *
 TGC AAG CTG GGA TGC AGA AGA ATT TGG TCT TCT TGG TTC TAC TGA GTG

Ala Ser Trp Asp Ala Glu Glu Phe Gly Leu Leu Gly Ser Thr Glu Trp>

1300 1310 1320 1330 1340
 * * * * * * *
~~GCG AGA GGA GAA TTC AAG ACT CCT TCA AGA GCG TGG CGT GGC TTA TAT~~

Ala Glu Glu Asn Ser Arg Leu Leu Gln Glu Arg Gly Val Ala Tyr Ile>

1350 1360 1370 1380 1390
 * * * * * * *
 TAA TGC TGA CTC ATC TAT AGA AGG AAA CTA CAC TCT GAG AGT TGA TTG

Asn Ala Asp Ser Ser Ile Glu Gly Asn Tyr Thr Leu Arg Val Asp Cys>

1400 1410 1420 1430 1440
 * * * * * * *
 TAC ACC GCT GAT GTA CAG CTT GGT ACA CAA CCT AAC AAA AGA GCT GAA

Thr Pro Leu Met Tyr Ser Leu Val His Asn Leu Thr Lys Glu Leu Lys>

1450 1460 1470 1480
 * * * * * * *
 AAG CCC TGA TGA AGG CTT TGA AGG CAA ATC TCT TTA TGA AAG TTG GAC

Ser Pro Asp Glu Gly Phe Glu Gly Lys Ser Leu Tyr Glu Ser Trp Thr>

FIGURE 55G

1490 1500 1510 1520 1530
 * * * * * * *
 TAA AAA AAG TCC TTC CCC AGA GTT CAG TGG CAT GCC CAG GAT AAG CAA

Lys Lys Ser Pro Ser Pro Glu Phe Ser Gly Met Pro Arg Ile Ser Lys>

1540 1550 1560 1570 1580
 * * * * * * *
~~ATT GGG ATC TGG AAA TGA TTT TGA GGT GTT CTT CCA ACG ACT TGG AAT~~

Leu Glv Ser Gly Asn Asp Phe Glu Val Phe Phe Gln Arg Leu Gly Ile>

1590 1600 1610 1620 1630
 * * * * * * *
 TGC TTC AGG CAG AGC ACG GTA TAC TAA AAA TTG GGA AAC AAA CAA ATT

Ala Ser Gly Arg Ala Arg Tyr Thr Lys Asn Trp Glu Thr Asn Lys Phe>

1640 1650 1660 1670 1680
 * * * * * * *
 CAG CGG CTA TCC ACT GTA TCA CAG TGT CTA TGA AAC ATA TGA GTT GGT

Ser Gly Tyr Pro Leu Tyr His Ser Val Tyr Glu Thr Tyr Glu Leu Val>

1690 1700 1710 1720
 * * * * * * *
 GGA AAA GTT TTA TGA TCC AAT GTT TAA ATA TCA CCT CAC TGT GGC CCA

Glu Lys Phe Tyr Asp Pro Met Phe Lys Tyr His Leu Thr Val Ala Gln>

FIGURE 55H

1730 1740 1750 1760 1770
 * * * * * * * * *
 GGT TCG AGG AGG GAT GGT GTT TGA GCT AGC CAA TTC CAT AGT GCT CCC

 Val Arg Gly Gly Met Val Phe Glu Leu Ala Asn Ser Ile Val Leu Pro>

 1780 1790 1800 1810 1820
 * * * * * * * * *
 TTT TGA TTG TCG AGA TTA TGC TGT AGT TTT AAG AAA GTA TGC TGA CAA

 Phe Asp Cys Arg Asp Tyr Ala Val Val Leu Arg Lys Tyr Ala Asp Lys>

 1830 1840 1850 1860 1870
 * * * * * * * * *
 AAT CTA CAG TAT TTC TAT GAA ACA TCC ACA GGA AAT GAA GAC ATA CAG

 Ile Tyr Ser Ile Ser Met Lys His Pro Gln Glu Met Lys Thr Tyr Ser>

 1880 1890 1900 1910 1920
 * * * * * * * * *
 TGT ATC ATT TGA TTC ACT TTT TTC TGC AGT AAA GAA TTT TAC AGA AAT

 Val Ser Phe Asp Ser Leu Phe Ser Ala Val Lys Asn Phe Thr Glu Ile>

 1930 1940 1950 1960
 * * * * * * * *
 TGC TTC CAA GTT CAG TGA GAG ACT CCA GGA CTT TGA CAA AAG CAA CCC

 Ala Ser Lys Phe Ser Glu Arg Leu Gln Asp Phe Asp Lys Ser Asn Pro>

FIGURE 551

1970 1980 1990 2000 2010
 * * * * * * *
 AAT AGT ATT AAG AAT GAT GAA TGA TCA ACT CAT GTT TCT GGA AAG AGC

Ile Val Leu Arg Met Met Asn Asp Gln Leu Met Phe Leu Glu Arg Ala>

2020 2030 2040 2050 2060
 * * * * * * *
 ATT TAT TGA TCC ATT AGG GTT ACC AGA CAG GCC TTT TTA TAG GCA TGT

Phe Ile Asp Pro Leu Gly Leu Pro Asp Arg Pro Phe Tyr Arg His Val>

2070 2080 2090 2100 2110
 * * * * * * *
 CAT CTA TGC TCC AAG CAG CCA CAA CAA GTA TGC AGG GGA GTC ATT CCC

Ile Tyr Ala Pro Ser Ser His Asn Lys Tyr Ala Gly Glu Ser Phe Pro>

2120 2130 2140 2150 2160
 * * * * * * *
 AGG AAT TTA TGA TGC TCT GTT TGA TAT TGA AAG CAA AGT GGA CCC TTC

Gly Ile Tyr Asp Ala Leu Phe Asp Ile Glu Ser Lys Val Asp Pro Ser>

2170 2180 2190 2200
 * * * * * * *
 CAA GGC CTG GGG AGA AGT GAA GAG ACA GAT TTA TGT TGC AGC CTT CAC

Lys Ala Trp Gly Glu Val Lys Arg Gln Ile Tyr Val Ala Ala Phe Thr>

2210 2220 2230 2240 2250
 * * * * * * *
 AGT GCA GGC AGC TGC AGA GAC TTT GAG TGA AGT AGC CTA AGA GGA TTC

Val Gln Ala Ala Ala Glu Thr Leu Ser Glu Val Ala

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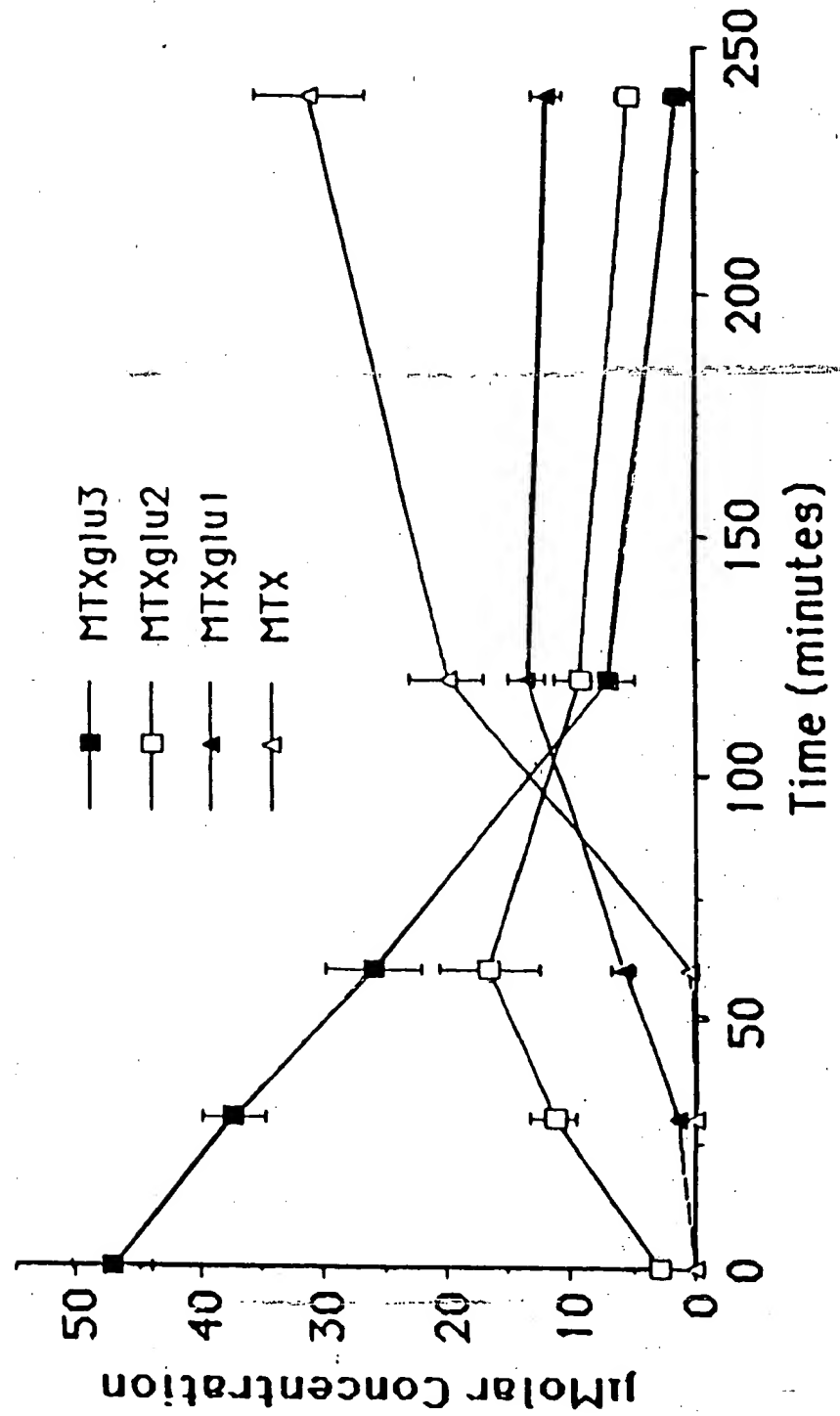
FIGURE 58J

2260 2270 2280 2290 2300
* * * * *
TTT AGA GAA TCC GTA TTG AAT TTG TGT GGT ATG TCA CTC AGA AAG AAT

~~2310~~ ~~2320~~ 2330 ~~2340~~ ~~2350~~
* * * * *
CGT AAT GGG TAT ATT GAT AAA TTT TAA AAT TGG TAT ATT TGA AAT AAA

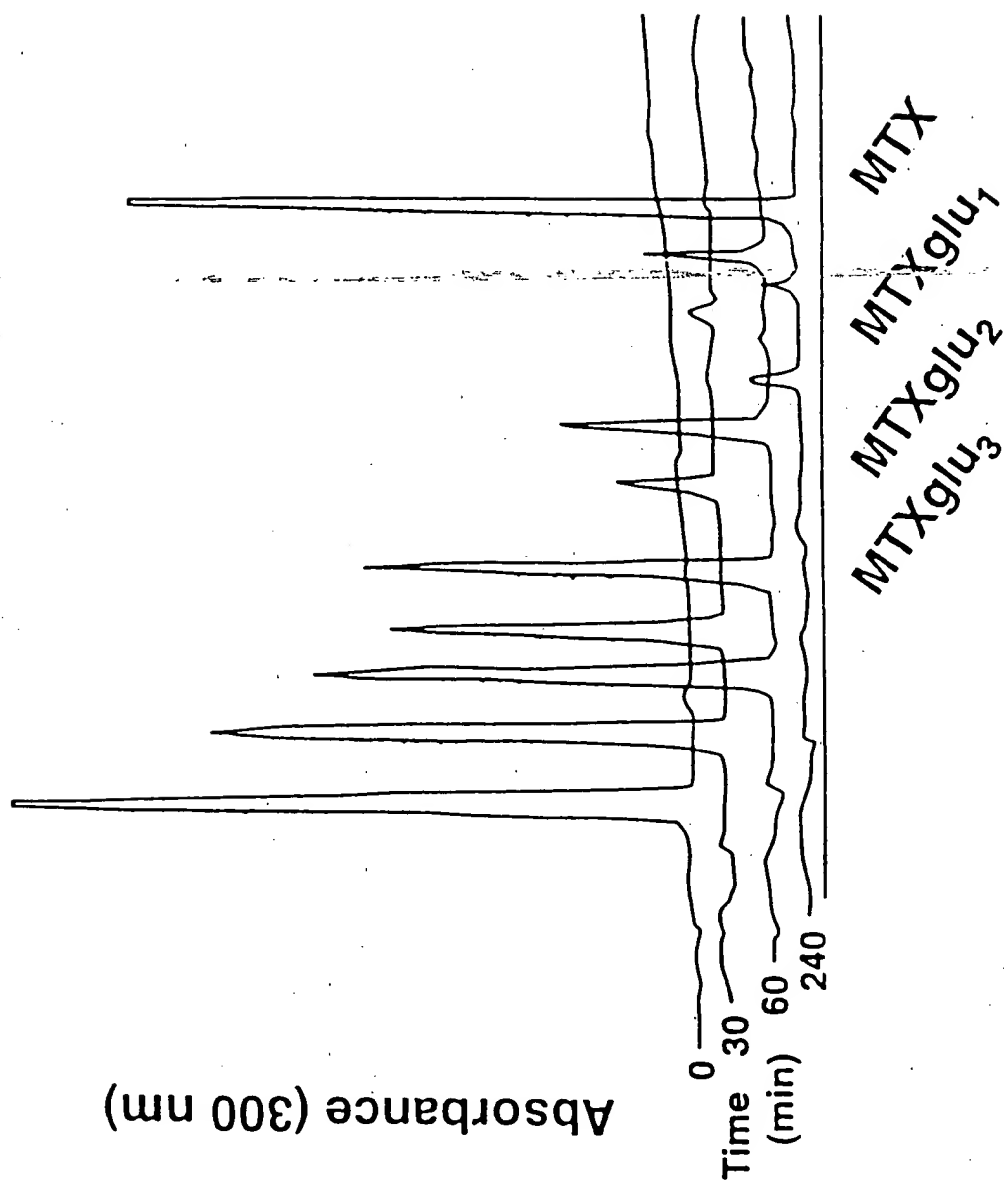
2360 2370 2380
* * * * *
GTT GAA TAT TAT ATA TAA AAA AAA AAA AAA AA

FIGURE 56



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FIGURE 57



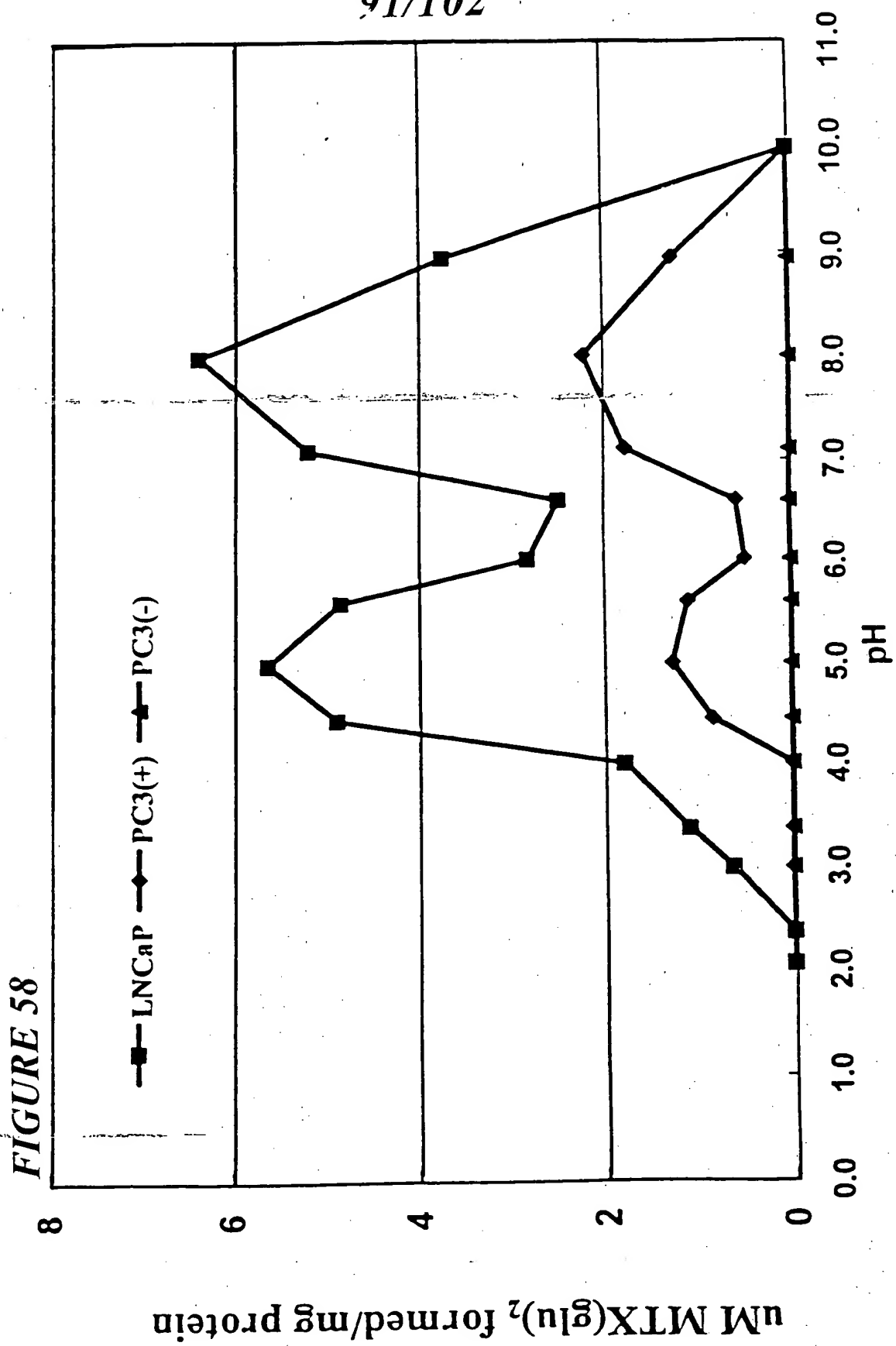
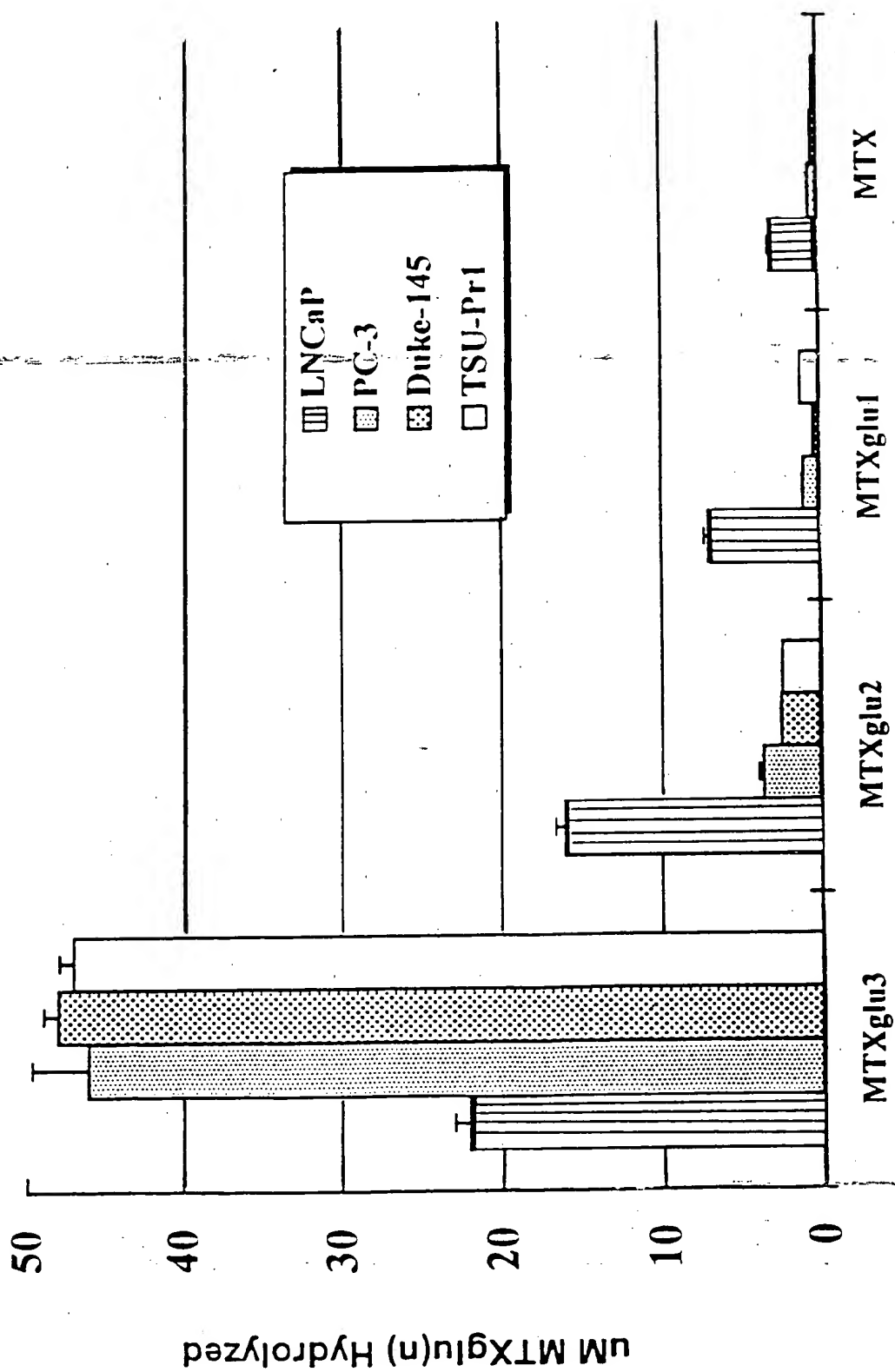
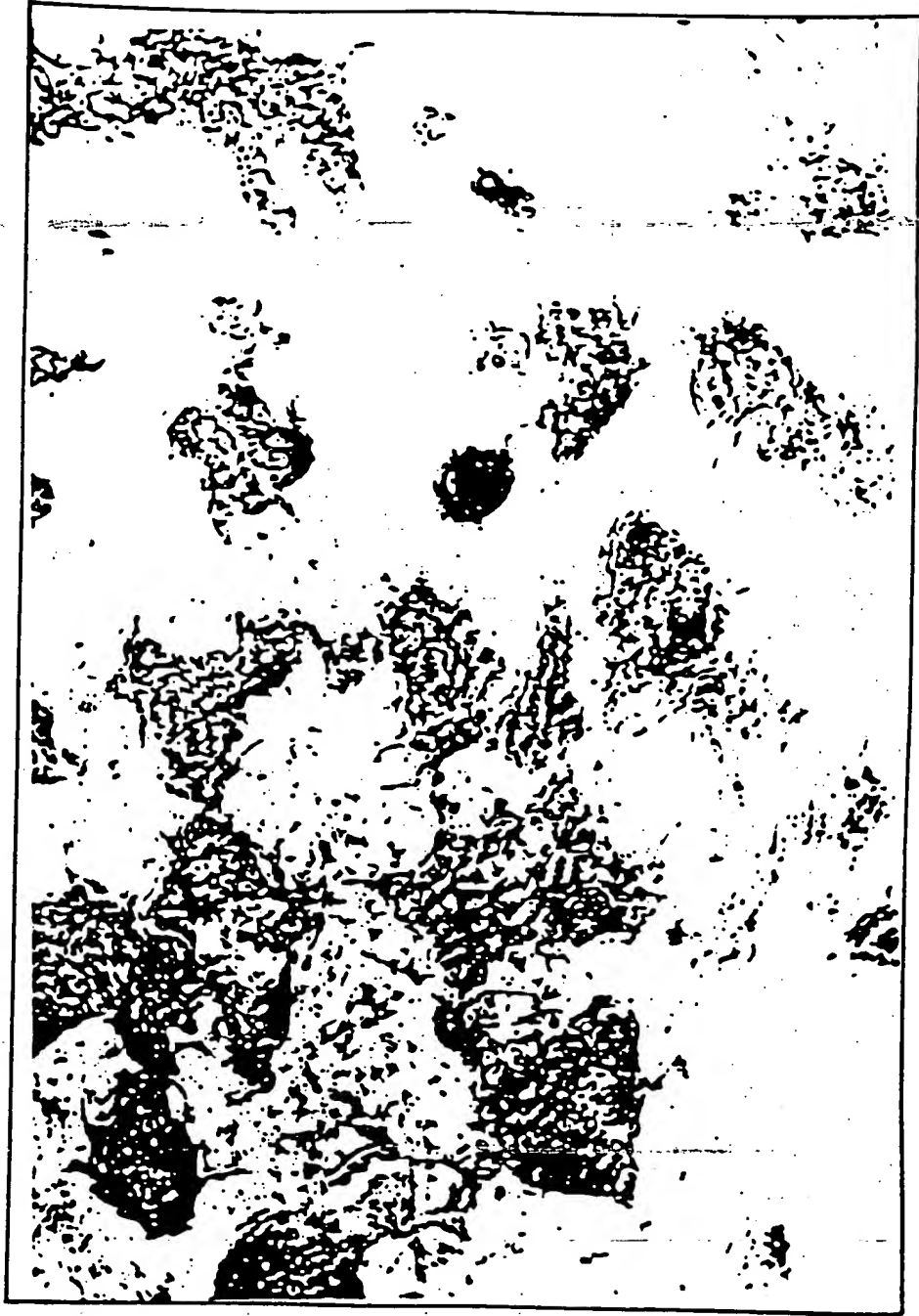


FIGURE 59



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FIGURE 60A



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FIGURE 60B



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FIGURE 60C

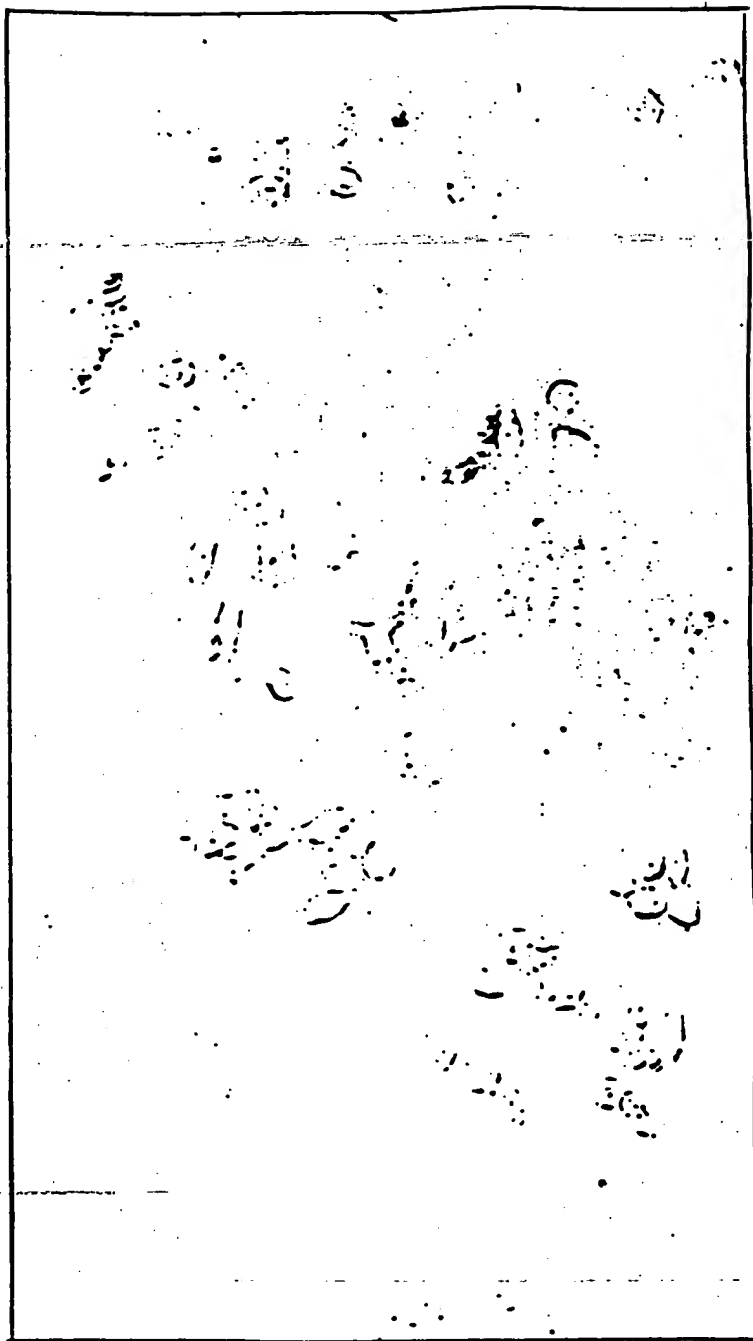


FIGURE 61

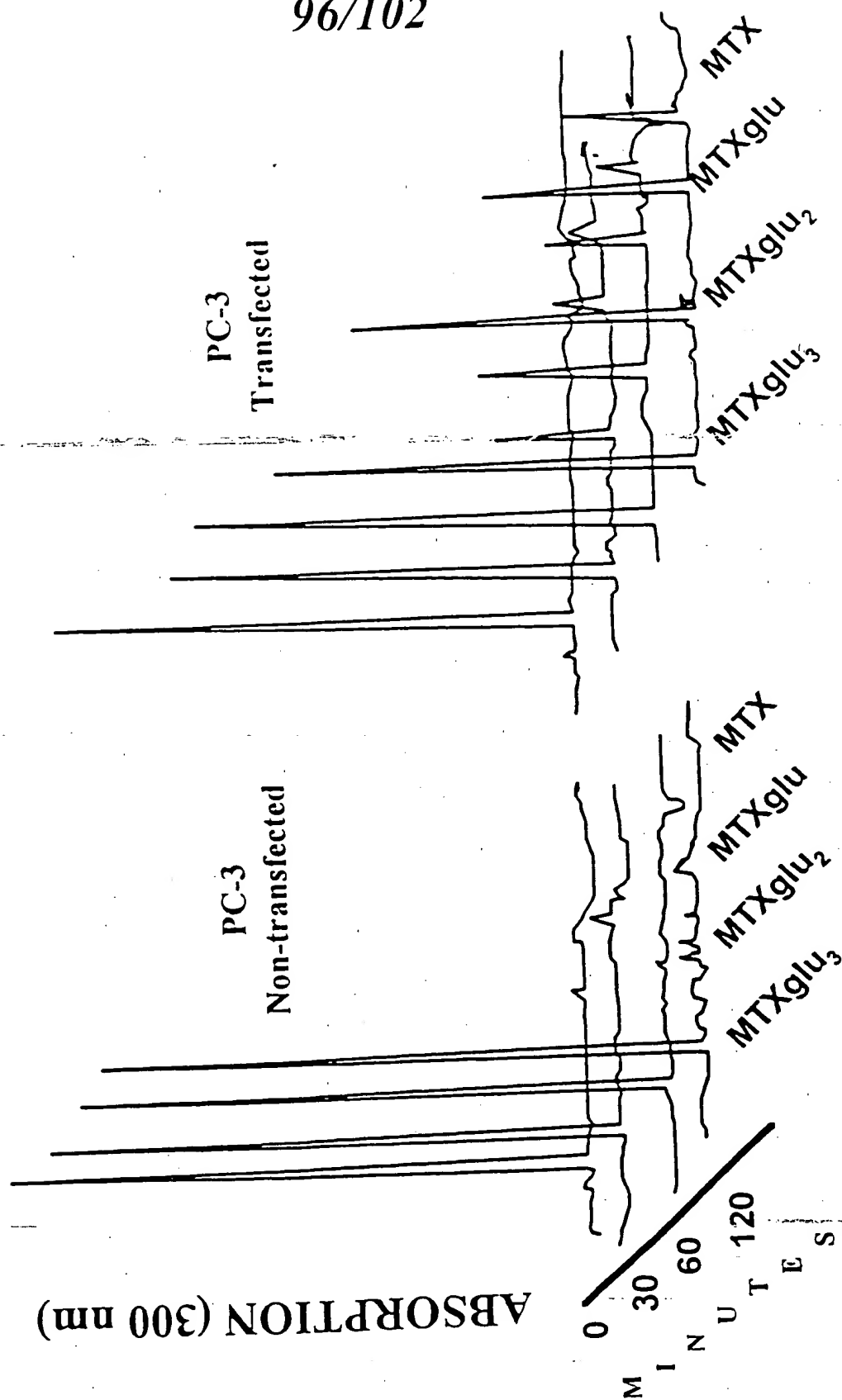


FIGURE 62

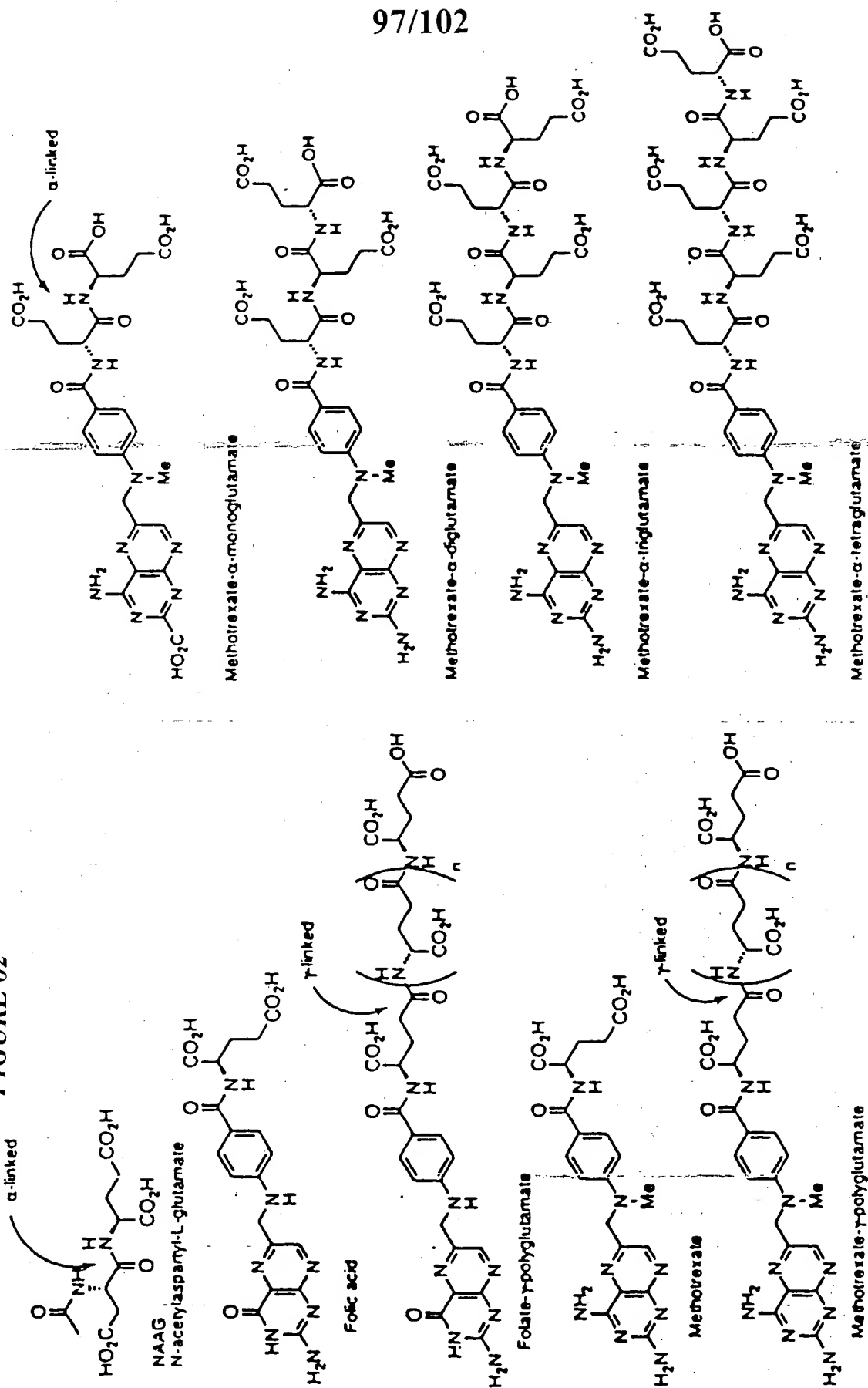


FIGURE 63A

Solid Phase Synthesis of Methotrexate α -polyglutamate Analogs

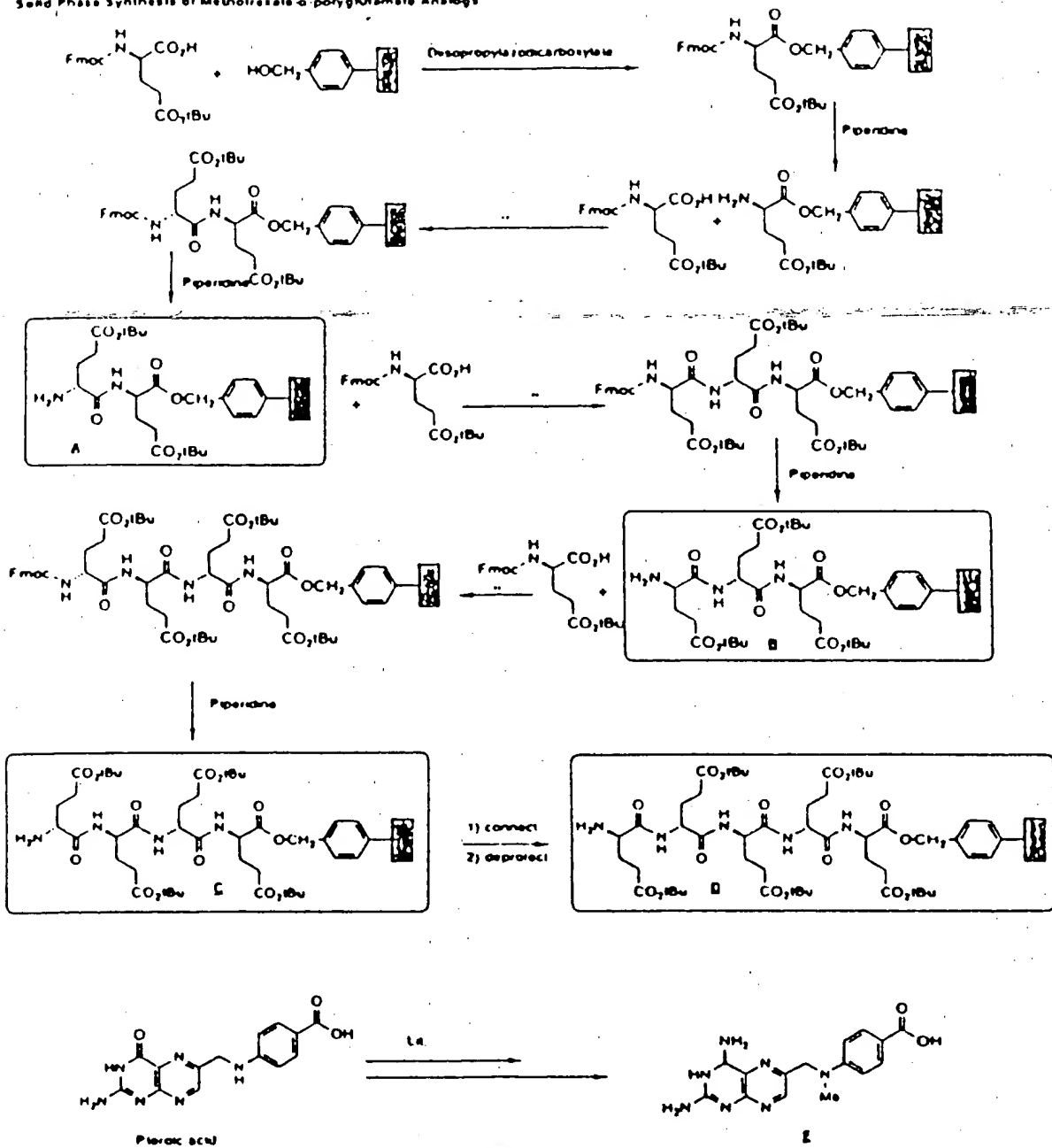


FIGURE 63B

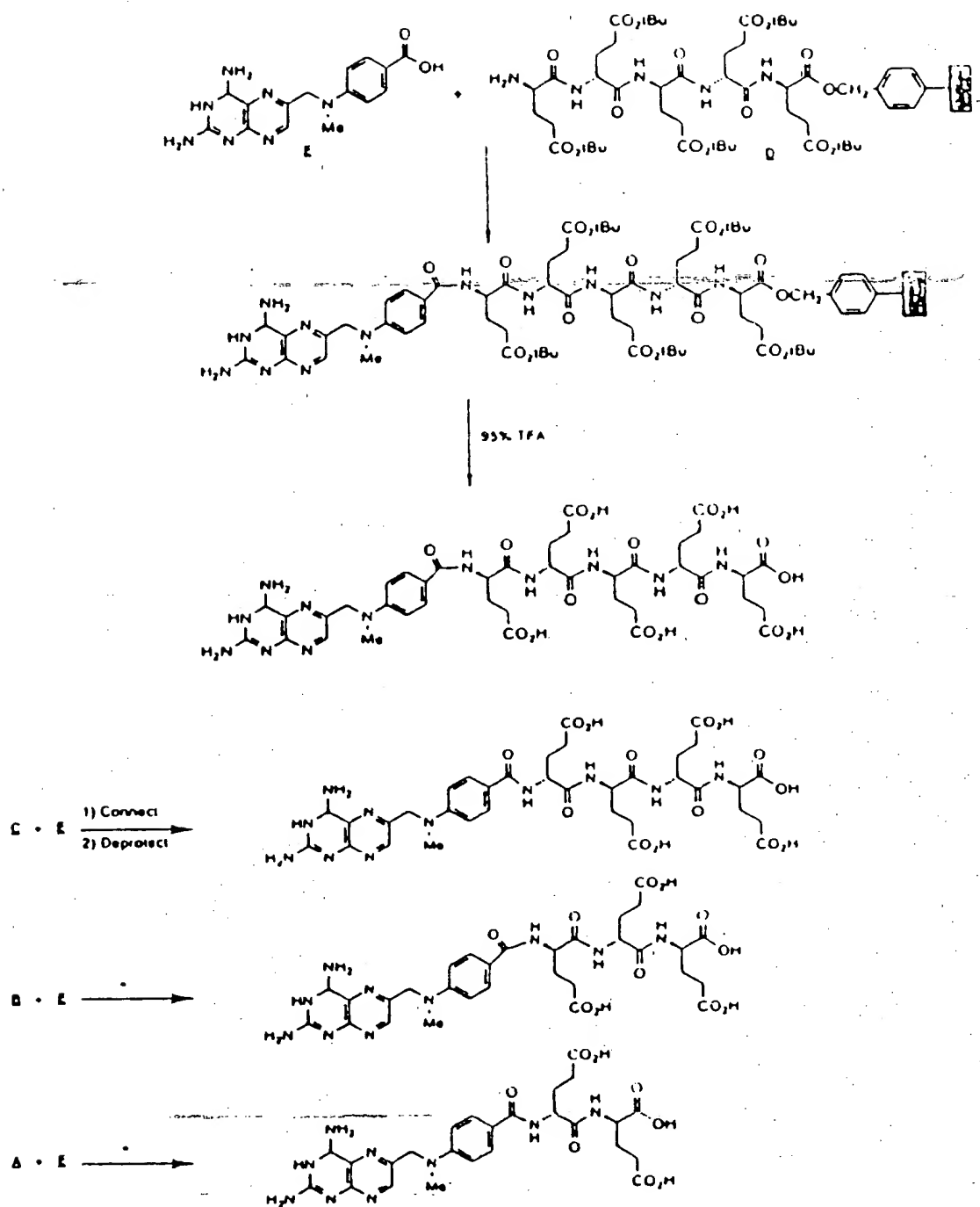


FIGURE 64

Sequence Analysis of microsatellite instability in PSM gene

<u>Sample</u>	<u>Sequence</u>	<u>PSM EXPRESSION (IMMUNO STAIN)</u>
Genomic	$T_9GC(TTTTG)_8(TTTG)_3T_7$	
LNCaP	$T_9GC(TTTTG)_6(TTTG)_3T_7$	positive
PC-3	$T_9GC(TTTTG)_8(TTTG)_3T_6$	negative
DU145	$T_{10}GC(TTTTG)_5(TTTG)_2T_7$	negative
T4 (tumor)	$T_{10}GC(TTTTG)_6(TTTG)_3T_7$	positive
N4 (paired normal)	$T_9GC(TTTTG)_6(TTTG)_3T_7$	positive

FIGURE 65

Genomic Organization of PSM Gene

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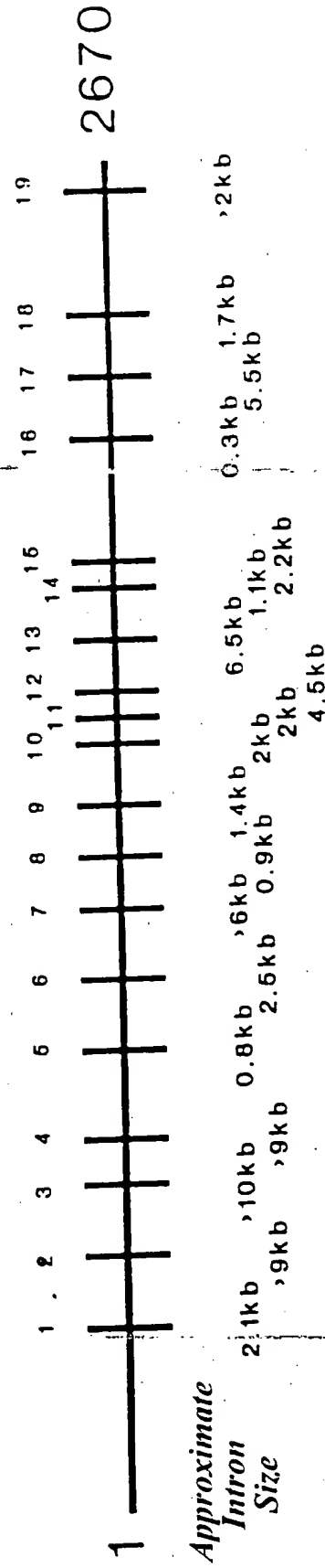
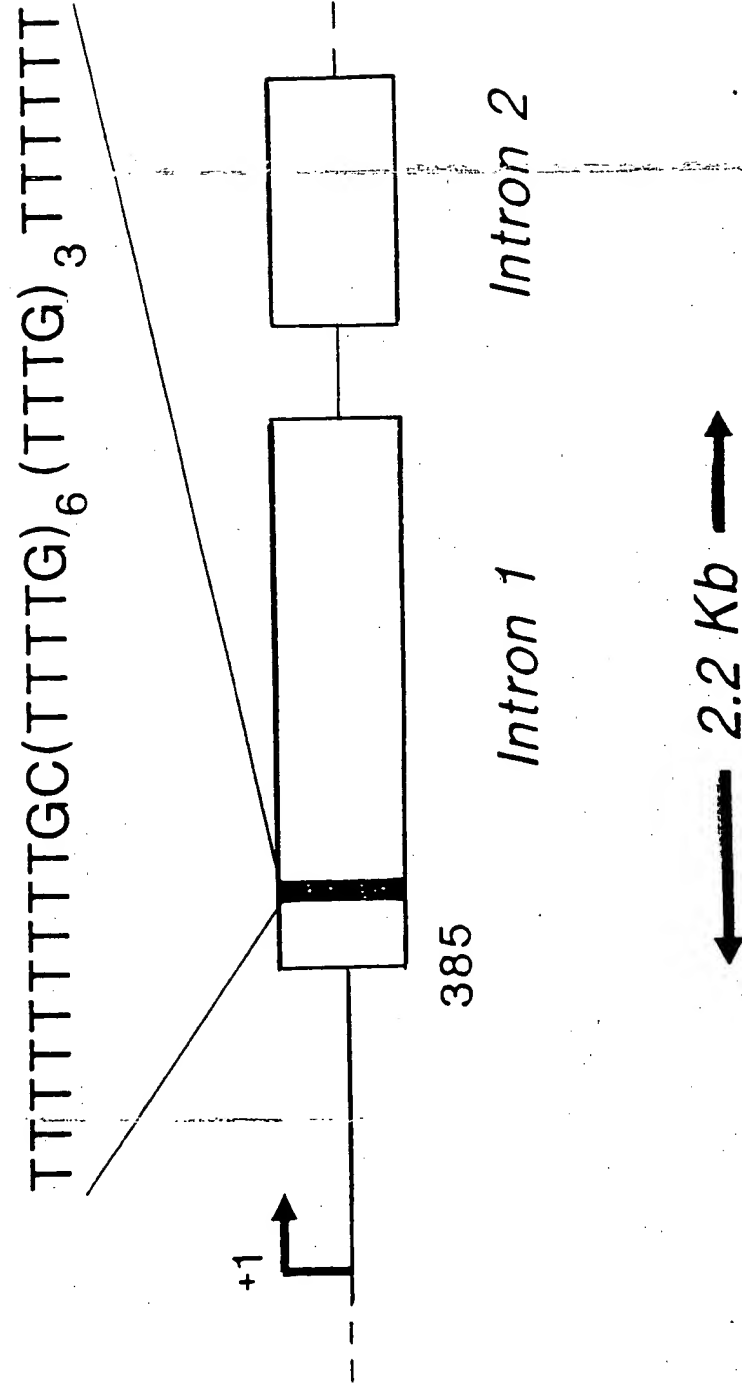


FIGURE 66

Location of microsatellite in PSM Gene

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Ron S. Israeli, Warren D. W. Heston, William
R. Fair, Ouathek Ouerfelli and John Pinto
Serial No. : NOT YET KNOWN
Filed : HEREWITH
For : PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES
THEREOF

1185 Avenue of the Americas
New York, New York 10036
July 2, 2003

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

PRELIMINARY AMENDMENT AND INFORMATION DISCLOSURE STATEMENT

Please amend the above-identified application as follows:

Amendments to the Specification:

Please amend the specification under the provisions of 37 C.F.R. §1.121 (revised Amendment format) as indicated below, with added matter indicated by underlining and deleted matter indicated by strikethrough.

Please amend the paragraph on page 91, lines 30-33 as follows:

--PSM is a type two membrane protein. Most type two membrane proteins are binding proteins, transport proteins or peptidases. Prostate Specific Membrane Antigen has activity as a carboxypeptidase and acts on both gamma linked or alpha linked amino acids which have acidic amino acids such as glutamate in the carboxy terminus.--

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Please amend the paragraph running from page 91, line 35 to page 92, line 7 as follows:

--Prostate specific membrane antigen is found in high concentration in the seminal plasma. When examining LNCaP cells, PSM antigen has enzymatic activity with N-acetylaspartylglutamate as a substrate and enzymatic action results in the release of, N-acetylaspartate and glutamic acid. In vitro translated PSM message also had this peptidase activity. Because PSM action will release glutamate, and because it is well known that the seminal fluid is highly enriched in its content of glutamic acid, the action of PSM antigen of endogenous protein/peptide substrates may be responsible for generating the glutamic acid present.--

On page 92, line 18, please add the following:

--Thus one skilled in this art would be able to design inhibitors to enhance the activity of the non degraded normal substrate if its increased level will have a biologic desired activity. Also biologic activity can be measured to see how it correlates with the level of message. Tissue may be examined for activity directly rather than indirectly using in-situ analysis or immunohistochemical probes. Because there is another gene highly similar on the other arm of chromosome 11 when isolated the expressed cloned genes can be used to determine what the substrate differences are and one may use those substrates for identification of PDM related activity, for example, in circulating cells when looking for metastases.

PSM specific substrates can be designed that could activate pro-drugs at the site of prostate tumor cells to kill those cells.--

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Please amend the paragraph on page 93, lines 1-14 as follows:

--Excitatory neurotransmission in the central nervous system (CNS) is mediated predominantly by glutamate receptors. Two types of glutamate receptors have been identified in the human CNS: metabotropic receptors, which serve G-protein coupled second messenger signaling systems, and ionotropic receptors, which serve as ligand gated ion channels. Ionotropic glutamate channels can increase the inward flow of ions such as calcium ions. This can result in the subsequent stimulation of nitric oxide, and nitric oxide modulation of a number of signaling pathways. Nitric oxide has been found to be a major signaling mechanism involved in cell growth and death, response to inflammation, smooth muscle cell contraction, etc. The presence of ionotropic glutamate receptors in human prostate tissue was investigated.--

Please amend the paragraph on page 93, lines 16-19 as follows:

--Methods: Detection of glutamate receptor expression was performed using anti-gluR2/3 and antigluR4 polyclonal antibodies and antibiotin immunohistochemical techniques in paraffin-embedded human prostate tissues. PSM antigen is a neurocarboxypeptidase that acts to release glutamate. In the CNS glutamate acts as a neurotransmitter by acting on glutaminergic ion channels and increases the flow of ions like calcium ions. One way the glutamate signal is transduced into cell activity is the activation of nitric oxide synthase, and nitric oxide synthase has recently been found to be present in human prostatic tissue. NO is a major signalling mechanism and is involved in control of cell growth and death, in response to inflammation, in smooth muscle cell contraction, etc. In the prostate much of the stroma is smooth muscle. It was discovered that the prostate is rich in glutaminergic receptors and we have begun to define this

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relationship. Stromal abnormalities are the key feature of BPH. Stromal epithelial interactions are of importance in both BPH and CaP. The other glutaminergic receptors through G proteins to change the metabolism of the cell. --

Please amend the paragraph running from page 93, line 29 to page 94, line 2 as follows:

--Discussion: PSM antigen is a neurocarboxypeptidase that acts to release glutamate from NAAG 1, also as a potential neurotransmitter. In the CNS glutamate acts as a neurotransmitter by acting on glutaminergic ion channels and increases the flow of ions such as calcium ions. One way the glutamate signal is transduced into cell activity is the activation of nitric oxide synthase, and nitric oxide synthase has recently been found to be present in human prostatic tissue. NO is a major signaling mechanism and is involved in control of cell growth and death, in response to inflammation, in smooth muscle cell contraction, etc.. In the prostate much of the stroma is smooth muscle. The prostate is rich in glutaminergic receptors. Stromal abnormalities are the key feature of BPH. Stromal epithelial interactions are of importance in both BPH and CaP. The other glutaminergic receptors through G proteins to change the metabolism of the cell. Glutamate can be produced in the cerebral cortex through the carboxypeptidase activity of the prostatespecific membrane antigen (PSMA). In this location, PSMA cleaves glutamate from acetyl-aspartyl-glutamate. Taken together, these observations suggest a function for PSMA in the human prostate; glutamate may be an autocrine and/or paracrine signalling molecule, possibly mediating epithelial-stromal interactions. Ionotropic glutamate receptors display a unique compartmental distribution in the human prostate. Distribution

The differential distribution of ionotropic glutamate receptor subtypes between the stromal and epithelial compartments of

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~~receptors~~ in the prostate has not been described. Prostate-specific membrane antigen(PSMA) has an analogous prostatic distribution, with expression restricted to the epithelial compartment. Basal cells are considered the precursor cell for the prostatic acinar and neuroendocrine cells of the prostate. Glutamate receptors may provide signaling functions in their interactions with the prostate stroma and acinar cells, and PSM may be involved in that interaction. Thus inhibition or enhancement of PSM activity could serve to modulate activity of the basal cells and prove to be a valuable aid for controlling basal cell function in the prostate.--

On page 94, line 21, please add the following:

--PSM carboxypeptidase may serve to process neuropeptide transmitters in the prostate. Neuropeptide transmitters are associated with the neuroendocrine cells of the prostate and neuroendocrine cells are thought to play a role in prostatic tumor progression. Interestingly, PSM antigen's expression is upregulated in cancer. Peptides known to act as prostatic growth factors such as TGF-a and bFGF, up regulate the expression of the antigen. TNF on the other hand downregulate PSM. TGF and FGF act through the mitogen activated signaling pathway, while TNF acts through the stress activated protein kinase pathway. Thus modulation of PSM expression is useful for enhancing therapy.--

Please amend the paragraph on page 94, lines 22-28 as follows:

--Because of PSM's carboxypeptidase-like activity and due to the fact that one of its substrates is the dipeptide N-acetyl-aspartyl glutamic acid, NAAG, which is one of the best substrates found to date to act as a neurotransmitter in the central nervous system, Aaltering PSM antigen function may also have beneficial actions outside the prostate. In the rat CNS a protein homology

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to PSM antigen was discovered and provides a rationale to consider prostate specific membrane antigen as a neurocarboxypeptidase. Abnormalities or Alterations in its function may occur in neurotoxic disorders such as epilepsy, or ALS, alzheimers, and multiple sclerosis. --

Please amend the paragraph on page 103, lines 2-21 as follows:

--Membrane-bound PSM antigen has pteroyl poly gamma-glutamyl carboxypeptidase (folate hydrolase) activity. Prostate specific membrane antigen was immuno precipitated from the prostate cancer cell line LNCaP and demonstrated it to be rich in folate hydrolase activity, with gamma-glutamated folate or polyglutamated methotrexate being much more potent inhibitors of the neuropeptidase activity than was quisqualate, which was the most potent inhibitor reported up to this time and consistent with the notion that polyglutamated folates may be the preferred substrate. Gamma-glutamyl hydrolase activity is also present in lysosomes of cells and these enzymes may be responsible for regulating the length of exogenous and endogenous folyl polyglutamate chain lengths. A characteristic difference between these two hydrolases is that the PSM enzyme exhibits substantial activity at pH values 7.5 to 8.0 in addition to having an acidic pH 4.5 to 5 optimum. Moderate levels of hydrolase activity are present within LNCaP cytosolic compartment and may represent the short intracellular fragment of this class II enzyme. This reflects an interesting situation in these cells where the majority of RNA codes for the membrane-bound enzyme that is localized extracellularly. The ratio of the mRNAs in these samples that code for the class II membrane and the cytosolic proteins is ten to one. In normal prostate tissue, the mRNA coding for the membrane protein is only one-tenth that of the cytosolic form.--

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Please amend the paragraph on page 105, lines 7-18 as follows:

--PSM folate hydrolase activity can possibly be used as a prodrug converting enzyme. Prodrugs may be generated which would activate at the site of the tumor such as N-phosphonoacetyl-1-aspartate-glutamate. PALglu is an inhibitor of the enzyme activity with NAAG as a substrate. In the normal prostate PSM is intracellular. In the transformed cell the majority of the protein and its attendance enzymatic activity is extracellular in location. It may be that as the enzymes associated with cell growth require the polyglutamated forms the cancer finds a way to remove PSM folate hydrolase from the interior by alternative splicing to an extracellular enzyme. PSM is a membrane protein and is found to predominate in cancer, but PSM' is likely a cytosolic protein which predominates in the normal condition.--

On page 105, line 24, please add the following:

-- For the cytotoxic drug methotrexate to be a tumor toxin it has to get into the cell and be polygamma-glutamated to be active, because polyglutamated forms serve as the enzyme substrates and because polyglutamated forms or toxins are also retained by the cell. Folate hydrolase is a competing reaction and de-glutamates methotrexate which then can diffuse back out of the cell. Cells that overexpose folate hydrolase activity are resistant to methotrexate. Prostate cancer has always been absolutely refractory to methotrexate therapy and this may explain why, since the prostate and prostate cancer has a lot of folate hydrolase activity.--

On page 108, line 4, please add the following:

--Penta-gamma-glutamyl-folate is a very potent inhibitor of activity (inhibition of the activity of the enzyme is with 0.5um Ki.) As penta-gamma-glutamyl-folate may also be a substrate and as

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folates have to be depolygammautamated in order to be transported into the cell, this suggests that this enzyme may also play a role in folate metabolism. Folate is necessary for the support of cell function and growth and thus this enzyme may serve to modulate folate access to the prostate and prostate tumor. The other area where PSM is expressed is in the small intestine. It turns out that a key enzyme of the small intestine that is involved in folate uptake acts as a gammacarboxypeptidase in sequentially proteolytically removing the terminal gamma-glutamyl group from folate. In the bone there is a high level of unusual gamma-glutamate modified proteins in which the gamma-glutamyl group is further carboxylated to produce gammacarboxyglutamate, or GLA. One such protein is osteonectin.

Using capillary electrophoresis pteroyl poly-gamma-glutamate carboxypeptidase (hydrolase) activity was investigated in membrane preparations from androgen sensitive human prostatic carcinoma cells (LNCaP). The enzyme immunologically cross-reacts with a derivative of an anti-prostate monoclonal antibody (7E11-C5) that recognizes prostate specific membrane (PSM) antigen. The PSM enzyme hydrolyzes gamma-glutamyl linkages and is an exopeptidase as it liberates progressively glutamates from methotrexate tri-glutamate (MTXGlu₃) and folate pentaglutamate (Pte Glu₅) with accumulation of MTX and Pte Glu respectively. The semi-purified membrane-bound enzyme has a broad activity from pH 2 to 10 and is maximally active at pH 4.0. Enzymatic activity was weakly inhibited by dithiothreitol (>0.2 mM) but not by reduced glutathione, homocysteine, or p-hydroxymercuribenzoate (0.05 mM). By contrast to LNCaP cell membranes, membranes isolated from androgen insensitive human prostate (TSU-Prl, Duke-145, PC-3) and estrogen-sensitive mammary adenocarcinoma (MCF-7) cells do not exhibit comparable hydrolase activity nor do they react with 7E11-C5. Thus, a folate hydrolase was identified in LNCaP cells that exhibits exopeptidase activity and is strongly expressed by these cells.

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PALA-Glutamate 3 was tested for efficacy of the prodrug strategy by preparing N-acetylaspartylglutamate, NAAG 1 (Figure 33). NAAG was synthesized from commercially available gamma-benzylaspartate which was acetylated with acetic anhydride in pyridine to afford N-acetylgamma-benzyl aspartate in nearly quantitative yield. The latter was activated as its pentafluorophenyl ester by treatment with pentafluorophenyltrifluoroacetate in pyridine at 0 deg.C for an hour. This activated ester constitutes the central piece in the preparation of compounds 1 and 4 (Figure 34). When 6 is reacted with epsilon-benzyl-L-glutamate in the presence of HOAT(1-hydroxy azabenzotriazole) in THF-DMF (tetrahydrofuran, N,N- dimethylformamide) at reflux for an overnight period and after removal of the benzyl protecting groups by hydrogenolysis (H2, 30 psi, 10% Pd/C in ethylacetate) gave a product which was identical in all respects to commercially available NAAG (Sigma).--

On page 109, line 33, please add the following:

--In addition, most if not all chemotherapies rely on one hypothesis; fast growing cells possess a far higher appetite for nutrients than normal cells. Hence, they uptake most of the chemotherapeutic drugs in their proximity. This is why chemotherapy is associated with serious secondary effects (weakening of the immune system, loss of hair,...) that sometimes put the patient's life in danger. A selective and effective drug that cures where it should without damaging what it shouldn't damage is embodied in representative structures 21 and 22. --

Please amend the paragraph on page 111, lines 17-24 as follows:

--In the latter, a compound like 27 when attached to a multiply charged dipeptide like NAAG, has no chance of crossing the blood brain barrier. In the former case, PSM homolog concentration in the small intestines is in the brush border and is low compared

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to that of PSM in the prostate cancer cells and is thus not likely to be exposed to prodrugs in the serum. In addition, one could enhance the selectivity of delivery of the prodrug by local injection in the prostate. Another image of this strategy could be formulated as follows. If prostate cancer were a war in which one needed a "smart bomb" to minimize the damage within the peaceful surroundings of the war zone, then 21 would be that "smart bomb". NAAG would be its guidance system, PSM would be the trigger, and 27 would be the warhead. --

Please amend the paragraph on page 111, lines 26-35 as follows:

--26 and its analogs are established active molecules that portray the activity of dynemycin A. Their synthesis is described in the literature. The total synthesis of optically active 27 has been described⁶. The synthetic scheme that for the preparation of 28 is almost the same as that of 27. However, they differ only at the position of the methoxy group which is meta to the nitrogen in the case of 28. This requires an intermediate of type 29 prepared by modification of the Myers' method. Compound 28 is perhaps the closest optically active analog to 26, and the activity of the latter is known and very high.--

Please amend the paragraph on page 112, lines 19-28 as follows:

--Intramolecular assisted hydrolysis of systems like N-acetylaspartyle is well documented in the literature. The aminoacid portion should facilitate the hydrolysis of such a linkage. In the event this would not work when NAAG is placed on the nitrogen, an alternative would be to attach NAAG to the oxygen giving rise to phenolic ester 22 which is per se labile and removable under milder conditions. PSM specific pro-drugs can be designed that could activate pro-drugs at the site of

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prostatic tumor cells to kill those cells. PSM specific substrates may also be used in the treatment of benign prostatic hyperplasia.--

In the claims:

Please amend as follows:

Please cancel claims 1-23 without prejudice or disclaimer.

Please add the following new claims to the application:

--24. (New) A method comprising administering an inhibitor of the neurocarboxypeptidase activity of prostate specific membrane antigen so as to inhibit release of glutamate by N-acetylaspartylglutamic acid (NAAG) hydrolysis.--

--25. (New) The method of claim 24, wherein the inhibitor is MTXglu₃, pteglu₅ or pABAglu₅.

REMARKS

In the present Preliminary Amendment, claims 1-23 of the application are cancelled without prejudice or disclaimer, and replaced by new claims 24-25.

Support for these new claims is found, inter alia, in the specification as follows: Claim 24: page 92, lines 22-30 and page 102, lines 29-36, and Claim 25: page 102, lines 34-36.

These new claims, 24-25, therefore raise no issue of new matter and it is respectfully requested that they be entered into the file of the application.

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Applicants note that new claims 24-25 embrace subject matter which is also claimed in U.S. Patent No. 6,413,948 issued July 2, 2002. As required by 35 U.S.C. §135(b), applicants are presenting claims to such subject matter not more than one year after the issue date of U.S. Patent No. 6,413,948. A copy of U.S. Patent No. 6,413,948 is attached hereto as Exhibit 1.

Information Disclosure Statement

In accordance with their duty of disclosure under 37 C.F.R. §1.56, applicants direct the Examiner's attention to the following reference which is listed on the PTO-1449 form attached hereto as Exhibit A. A copy of this reference is attached hereto as Exhibit 1.

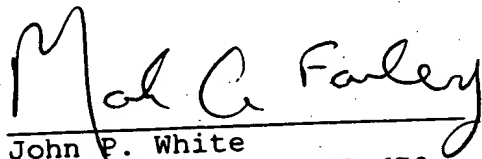
U.S. Patent No. 6,413,948, issued July 2, 2002 to Slusher, et al. (Exhibit 1).

If a telephone conference would be of assistance in advancing the progress of this application, applicants' undersigned attorney invites the Examiner to telephone either of them at the number provided below.

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No fee is deemed necessary with the filing of this Preliminary Amendment. However, if any fee is due, authorization is hereby given to charge the required fee to Deposit Account No. 03-3125.

Respectfully submitted,



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Applicants: Ron S. Israeli et al.
U.S. Serial No.: 08/894,583
Filed: February 23, 1998

Pending Claims 30-32

30. A method of detecting expression of an alternatively spliced prostate specific membrane antigen in a cell or tissue, which alternatively spliced prostate specific membrane antigen consists essentially of consecutive amino acids, the amino acid sequence of which is set forth in SEQ ID NO:128 beginning with methionine at position number 58 and ending with alanine at position number 750, the nucleic acid encoding the alternatively spliced prostate specific membrane antigen having an intron splice site located between the G and T nucleotides at positions 114 and 115, respectively, as set forth in SEQ ID NO:1, wherein a splice at said site results in formation of an exon-exon junction characteristic of said alternatively spliced prostate specific membrane antigen, said method comprising:

- (1) contacting mRNA obtained from the cell or tissue with a detectable nucleic acid of at least 15 nucleotides in length which specifically hybridizes across said exon-exon junction; and
- (2) determining whether the detectable nucleic acid hybridizes to the mRNA,

wherein the presence of the detectable nucleic acid hybridized to the mRNA indicates expression of alternatively spliced prostate specific membrane antigen in

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the cell or tissue.

31. The method of claim 30, wherein the detectable nucleic acid is labeled with a detectable label.

32. The method of claim 31, wherein the detectable label is a radioisotope or fluorescent dye.

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U.S. Serial No.: 10/751,346
Filed: January 2, 2004

Pending Claims 21-58

21. A method of ablating or killing normal, benign hyperplastic, and cancerous prostate epithelial cells comprising: providing a biological agent which binds to an outer membrane domain of prostate specific membrane antigen and contacting said cells with the biological agent under conditions effective to permit both binding of the biological agent to the outer membrane domain of the prostate specific membrane antigen and ablating or killing of said cells.
22. A method according to claim 21, wherein the biological agent is an antibody or ligand.
23. A method according to claim 21, wherein said contacting is carried out in a living mammal and comprises: administering the biological agent to the mammal under conditions effective to permit both binding of the biological agent to the outer membrane domain of the prostate specific membrane antigen and killing of said cells.
24. A method according to claim 23, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously or intramuscularly.

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25. A method according to claim 22, wherein an antibody is used in carrying out said method, the antibody being selected from the group consisting of a monoclonal antibody and a polyclonal antibody.
26. A method according to claim 22, wherein the ligand is used in carrying out said method.
27. A method according to claim 21, wherein the biological agent is bound to a substance effective to kill or ablate said cells upon binding of the biological agent to the outer membrane domain of the prostate specific membrane antigen of said cells.
28. A method according to claim 27, wherein the substance effective to kill said cells is a cytotoxic agent.
29. A method according to claim 28, wherein the cytotoxic agent is selected from the group consisting of a drug, a toxin, a radioactive substance, a chemotherapeutic, an enzyme and molecules of fungal, viral and bacterial origin.
30. A method according to claim 21, wherein the biological agent is in a composition further comprising a physiologically acceptable carrier, diluent, or stabilizer.
31. A method according to claim 21, wherein the biological agent is in a composition further comprising a

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pharmaceutically acceptable carrier, diluent, or stabilizer.

32. A method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells or a portion thereof in a biological sample comprising: providing a biological agent which binds to an outer membrane domain of prostate specific membrane antigen, wherein the biological agent is bound to a label effective to permit detection of said cells or a portion thereof upon binding of the biological agent to said cells or a portion thereof; contacting the biological sample with the biological agent having a label under conditions effective to permit binding of the biological agent to the outer membrane domain of the prostate specific membrane antigen of any of said cells or a portion thereof in the biological sample; and detecting a presence of any of said cells or a portion thereof in the biological sample by detecting the label.
33. A method according to claim 32, wherein the biological agent is an antibody or ligand.
34. A method according to claim 32, wherein said contacting is carried out in a living mammal and comprises: administering the biological agent to the mammal under conditions effective to permit binding of the biological agent to the outer membrane domain of the prostate specific

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membrane antigen of any of said cells or a portion thereof in the biological sample.

35. A method according to claim 34, wherein the label is a radioactive substance.
36. A method according to claim 34, wherein the biological sample is a mammal's prostatic tissue.
37. A method according to claim 34, wherein said detecting is carried out after a prostatectomy.
38. A method according to claim 34, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously or intramuscularly.
39. A method according to claim 33, wherein an antibody is used in carrying out said method, said antibody being selected from the group consisting of a monoclonal antibody and a polyclonal antibody.
40. A method according to claim 33, wherein a ligand is used in carrying out said method.
41. A method according to claim 32, wherein the label is selected from the group consisting of a fluorescent label and a radioactive label.

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42. A method according to claim 32, wherein the biological agent is in a composition further comprising a physiologically acceptable carrier, diluent, or stabilizer.
43. A method according to claim 32, wherein the biological agent is in a composition further comprising a pharmaceutically acceptable carrier, diluent, or stabilizer.
44. A method according to claim 32, wherein said contacting is carried out in a sample of serum or urine.
45. An isolated biological agent which binds to an outer membrane domain of prostate specific membrane antigen.
46. An isolated biological agent according to claim 45, wherein said isolated biological agent is an isolated antibody or ligand.
47. An isolated biological agent according to claim 46, wherein the isolated biological agent is an antibody selected from the group consisting of a monoclonal antibody and a polyclonal antibody.
48. An isolated biological agent according to claim 46, wherein the isolated biological agent is a ligand.

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49. An isolated biological agent according to claim 45, wherein the biological agent is bound to a cytotoxic agent.
50. An isolated biological agent according to claim 49, wherein the cytotoxic agent is selected from the group consisting of a drug, a toxin, a radioactive substance, a chemotherapeutic, and molecules of fungal, viral and bacterial origin.
51. A composition comprising: a biological agent according to claim 49 and a physiologically acceptable carrier, diluent, or stabilizer mixed with the biological agent.
52. A composition comprising: a biological agent according to claim 49 and a pharmaceutically acceptable carrier, diluent, or stabilizer mixed with the biological agent.
53. An isolated biological agent according to claim 45, wherein said biological agent is bound to a label.
54. An isolated biological agent according to claim 53, wherein the label is selected from the group consisting of a fluorescent label, a radioactive label and an immunohistochemical probe.
55. An isolated biological agent according to claim 45, wherein said biological agent is bound to a biologically active enzyme.

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56. A composition comprising: a biological agent according to claim 53 and a physiologically acceptable carrier, diluent, or stabilizer mixed with the biological agent.
57. A composition comprising: a biological agent according to claim 53 and a pharmaceutically acceptable carrier, diluent, or stabilizer mixed with the biological agent.
58. A hybridoma cell line that produces a monoclonal antibody which binds to an outer membrane domain of prostate specific membrane antigen.